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Road Effects on Vertebrates: From Rabbit Abundance to Tredator Habitat Use and Mortality

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A mi tía Marian,

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Chapter 1

Introducción

El aumento de la población humana y, por tanto, de las actividades asociadas con ésta, genera un impacto en medio cada vez mayor. El 83% de la superficie terrestre ya estaba bajo la influencia de alguna actividad humana en el año 2002 (Sanderson et al. 2002). La alteración humana del medio se considerada la principal causa de la sexta extinción masiva de especies en la historia y causa grandes cambios en la distribución de los organismos (Stuart Chapin III et al. 2000). La pérdida de biodiversidad a su vez altera el funcionamiento de los ecosistemas y repercute a su vez negativamente en la calidad de vida de las poblaciones humanas, por lo que en la actualidad se considera una prioridad minimizar tales alteraciones (Stuart Chapin III et al. 2000, Foley et al. 2005).

Una de las principales causas de pérdida de biodiversidad es la alteración del hábitat natural (Vitousek et al. 1997). Las infraestructuras lineales y, especialmente las carreteras, son una de las principales causas de fragmentación y degradación del hábitat en el mundo (van der Ree et al. 2011). Las carreteras

se extienden por los paisajes y pese a su pequeña superficie, sus efectos alcanzan áreas de gran extensión. Por ejemplo, el 80% de la superficie terrestre de Estados Unidos se encuentra a apenas 1 km de una carretera (Riitters and Wickham 2003) y en la mayoría de países europeos la densidad de carreteras es mayor que en EE.UU (Dulac 2013). Además, en los próximos años se prevé un considerable aumento de la red viaria global, un 60% para el año 2050, especialmente en los países en vías de desarrollo (Dulac 2013). Las carreteras generan muchos efectos nocivos sobre la fauna de los alrededores. Para todos los grupos de vertebrados, las redes de transporte se encuentran entre las 10 principales amenazas para su conservación (IUCN 2015). Pero no sólo las carreteras tienen efectos por sí mismas, sino que además están íntimamente ligadas a otras de las principales amenazas: el desarrollo urbanístico, las molestias por turismo a la fauna, el desarrollo de actividades recreativas, o la dispersión de especies exóticas (Trombulak and Frissell 2000, Forman et al. 2003, IUCN 2015). El papel central que juegan las carreteras como agentes directos o como facilitadores de otras actividades las convierte en una prioridad para la conservación de especies.

A la vez que aumentan los impactos, aumenta la preocupación por mitigar los efectos del tráfico sobre los ecosistemas (Fahrig and Rytwinski 2009). En los últimos años, se ha desarrollado una preocupación creciente por los efectos de la red de infraestructuras viarias sobre fauna del entorno . Como ejemplo, al introducir en el buscador “Scopus” las palabras clave “road+mortality”, en el año 2000 se recuperan 22 publicaciones dentro de los campos de la biología y las ciencias ambientales, en el año 2010 son 53 publicaciones, y en el año 2014 son 93 publicaciones.

Para que la mitigación sea efectiva, un primer paso necesario sobre el que

construir las medidas correctoras que permitan reducir los impactos negativos es el conocimiento y comprensión de los efectos de las carreteras. En este contexto se enmarca la presente tesis.

1.1 Efectos de las carreteras sobre la fauna

Las carreteras generan diversos efectos sobre la fauna de su entorno, siendo los principales la mortalidad, la destrucción de hábitat, la alteración y degradación del hábitat del entorno, el favorecimiento de la dispersión de especies invasoras y la fragmentación de poblaciones y el efecto barrera.

1.1.1 Mortalidad

La mortalidad por atropello es el efecto más visible de las carreteras sobre la fauna y uno de las principales causas de mortalidad derivadas de actividades humanas (Forman and Alexander 1998, Trombulak and Frissell 2000, Underhill and Angold 2000). Afecta a muchas especies de vertebrados (Gunson et al. 2011) y puede ser un riesgo para la supervivencia de algunas poblaciones mayor que la fragmentación o el efecto barrera (Jackson and Fahrig 2011). Se estima que un millón de vertebrados por día mueren atropellados en las carreteras de Estados Unidos (Forman and Alexander 1998).

Los atropellos se relacionan con factores propias de las carreteras y del paisaje, así como con factores propios de la ecología y comportamiento de las especies (Forman et al. 2003). Carreteras con mayores niveles de tráfico y mayor límite de velocidad se relacionan con altas tasas de atropellos (Lodé 2000, Saeki and Mac-

donald 2004, Farmer and Brooks 2012), al igual, que aquéllas que atraviesas áreas de hábitat favorable o corredores naturales (Forman et al. 2003, Grilo et al. 2011). Por otro lado, especies que no evitan la superficie de las carreteras o esquivan a los vehículos también están expuestas a una mayor probabilidad de atropello (Jaeger et al. 2005). Por último, especies que se ven atraídas a la carretera por algún recurso se exponen a un mayor riesgo de colisión (Forman et al. 2003).

La mortalidad afecta principalmente a poblaciones de especies que se encuentran en baja densidad y con bajas tasas reproductivas (Rytwinski and Fahrig 2012). Este es el caso de los carnívoros, de los que varias especies cuentan con poblaciones sometidas a altas mortalidades por atropello, entre ellos, el lince ibérico, el tejón, el oso negro o la pantera de Florida (Maehr et al. 1991, Ferreras et al. 1992, Brandenburg 1996, Clarke et al. 1998).

1.1.2 Destrucción de hábitat

Éste es el efecto más directo de las carreteras. La destrucción de hábitat por el cambio de uso del suelo reduce el espacio disponible para las especies con requerimientos propios de hábitats, siendo uno de los principales problemas de conservación en la actualidad (Foley et al. 2005). La construcción de carreteras destruye los hábitats de dos formas, directamente por los trabajos de construcción y asfaltado de superficies, e indirectamente, al interrumpir arroyos y cambiar los flujos naturales, lo que a veces desemboca en la destrucción de humedales (Trombulak and Frissell 2000, Forman et al. 2003).

1.1.3 Alteración del Hábitat

Además de la destrucción directa del hábitat, las carreteras también alteran la calidad del hábitat de los alrededores, generando un efecto borde en su entorno que reduce el hábitat original de las especies (Coffin 2007). Las molestias asociadas al tráfico, como un incremento del ruido, polvo, luz o polución, entre otras (Spellerberg 1998), afectan a las poblaciones silvestres del entorno y provocan respuestas de estrés, que generalmente se ven reflejadas en baja tasa reproductiva y menores abundancias (Forman et al. 2003). Aunque las carreteras son infraestructuras que generalmente ocupan sólo unos metros de terreno, el efecto asociado de degradación y alteración del hábitat del entorno puede extenderse varios kilómetros para aquéllas con mayores niveles de tráfico (Van der Zande et al. 1980, Reijnen et al. 1995).

Sin embargo, algunas especies responden positivamente a los hábitats próximos a carreteras, como los pequeños mamíferos que medran en los márgenes con vegetación (Bennett 1990, Bellamy et al. 2000, Brock and Kelt 2004), o algunas aves que seleccionan zonas próximas a las carreteras para anidar o por la disponibilidad de posaderos (Forman et al. 2003, Morelli 2011). En general, la proliferación de pequeños vertebrados está relacionada con el manejo al que es sometida de la vegetación de los márgenes (Forman and Alexander 1998) y es más marcada en aquellos márgenes que se encuentran delimitados por vallas que impiden el acceso de ungulados (Forman et al. 2003).

1.1.4 Invasión

Por último, al facilitar el acceso a nuevos lugares y disminuir el tamaño de las poblaciones naturales, las carreteras favorecen la dispersión de especies exóticas (Trombulak and Frissell 2000). Dentro de los animales que pueden utilizar los

márgenes de las carreteras como hábitat o corredor, son generalmente especies generalistas (Coffin 2007). Dichas especies son capaces de explotar condiciones ecológicas muy diversas, incluyendo los márgenes de carreteras (Forman and Alexander 1998), y generalmente son capaces de persistir y extenderse a través del paisaje usando estos márgenes. Entre las especies generalistas, se encuentra el caso particular de las especies invasoras, relacionadas con el declive de especies locales, cuyo avance se ha relacionado con las carreteras (Huey 1941, May and Norton 1996, Gurevitch and Padilla 2004, Hulme 2009).

1.1.5 Fragmentación y efecto barrera

Uno de los principales efectos de las carreteras es la fragmentación y la pérdida de conectividad (Carr et al. 2002, Coffin 2007). Las carreteras dividen el hábitat en parches más pequeños, separando poblaciones continuas en subpoblaciones relativamente aisladas (Forman and Alexander 1998). Además, los atropellos o la evitación de la carretera por algunas especies genera un efecto barrera que reduce la conectividad entre las poblaciones fragmentadas (Forman et al. 2003). La menor conectividad da lugar a poblaciones pequeñas, que están sometidas a mayores fluctuaciones y a un menor flujo de inmigrantes y, por tanto, a un mayor riesgo de extinción (Mader 1984, Andrews 1990, Forman and Alexander 1998). Además, la falta de conectividad es un problema para la supervivencia de especies que requieren de varios hábitats durante su ciclo vital, como anfibios que han de cruzar carreteras para llegar a las charcas de reproducción (Carr and Fahrig 2001). En ambos casos, la falta de conectividad se puede traducir en una menor viabilidad poblacional (Forman et al. 2003).

Como consecuencia de todos estos efectos, hay especies que se ven sometidas a impactos negativos por las carreteras, mientras que otras parecen ser beneficiadas. Sin embargo, la relación no está equilibrada. Fahrig y Rytwinski (2009) en una revisión de la literatura encontraron que los efectos negativos de las carreteras son 5 veces más frecuentes que los positivos. Aunque los efectos más llamativos y estudiados de las carreteras han sido aquéllos relacionados con la mortalidad por atropello y con la fragmentación de poblaciones, la alteración del hábitat puede tener serias repercusiones en el ecosistema. Mientras que algunas especies consiguen alcanzar altas densidades junto a las, otras ven sus poblaciones reducidas (Fahrig and Rytwinski 2009, Benítez-López et al. 2010).

Entre las poblaciones de vertebrados que aumentan su densidad junto a las carreteras se encuentran los pequeños mamíferos, mientras que entre las especies afectadas negativamente se encuentran los depredadores (Rytwinski and Fahrig 2012). Además de los efectos sobre especies concretas, en los que se centran la mayoría de estudios, a la hora diseñar medidas correctoras o preventivas eficaces es importante tener en cuenta que las especies se encuentran en una red interconectada, de forma que el resultado final no será la reacción pura de la especie a la infraestructura, sino la suma los efectos de la carretera al resto de condicionantes que afectan al uso del hábitat de las especies. En este caso, ambos tipos de especies afectadas se encuentran íntimamente relacionadas a través de las relaciones depredador-presa.

1.2 Alteración del hábitat e interacciones depredador-presa

Entre las especies que proliferan en los márgenes (o taludes) de carreteras o los

usan de forma habitual como corredores, destacan los pequeños mamíferos. Algunas especies de ratones y de topillos tienden a ser más abundantes en márgenes de carretera que conservan vegetación que en la matriz del paisaje o a desplazarse regularmente usando los márgenes de carreteras, especialmente cuando las carreteras atraviesan en zonas alteradas (Bennett 1990, Bellamy et al. 2000, de Redon et al. 2015). Este fenómeno también se observa en las carreteras de la Península Ibérica, donde ratones de campo (Apodemus sylvaticus), ratones morunos (Mus spretus) o musarañas (Crocidura russula) tienden a estar en altas densidades junto a carreteras (Sabino (Sabino-Marques and Mira 2011, Ruiz-Capillas et al. 2013b). También el conejo europeo (Oryctolagus cuniculus) se señala como una de las especies que podrían estar beneficiándose de los márgenes de las carreteras y alcanzando densidades en ellos mayores que en el paisaje (Bautista et al. 2004, Barrientos and Bolonio 2009).

Los márgenes de carreteras presentan varias ventajas para ratones y conejos. En ellos se pueden dar buenas condiciones de microhábitat por la estructura de la vegetación (Bellamy et al. 2000). Además, en carreteras que cuentan con una valla perimetral, los taludes de las carreteras quedan fuera del acceso de humanos y grandes herbívoros, reduciendo las molestias y la caza (Bautista et al. 2004, Ascensão et al. 2012). En zonas agrícolas o con escaso suelo apto para construcción de madrigueras, los taludes de carreteras suponen un lugar seguro y estable para la construcción de aquéllas, que en los campos serían demolidas por el arado de la tierra (Calvete et al. 2004, Gea-Izquierdo et al. 2005).

Al habitar cerca del tráfico, estas poblaciones se encuentran sometidas a la mortalidad por atropello (Adams and Geis 1983, Meunier et al. 1999, Ruiz-Capillas et al. 2015). No obstante, las propias características de las especies le pro-

porcionan una ventaja a nivel poblacional que les permite mantener e, incluso, aumentar sus poblaciones. Son especies con altas tasas reproductivas que compensan la mortalidad y con pequeñas áreas de campeo, por lo que no se exponen frecuentemente al tráfico (Fahrig and Rytwinski 2009).

El conejo europeo es una presa clave en el ecosistema mediterráneo, formando parte de la dieta de más 30 especies de depredadores (Delibes-Mateos et al. 2008b), desde especialistas como el lince ibérico (Delibes 1980) a generalistas como el zorro rojo, que selecciona conejos cuando se encuentran en altas densidades (Delibes-Mateos et al. 2008a). Sin embargo, el hecho de que los conejos se establezcan en los taludes puede generar efectos en cascada en el ecosistema. Así, en el caso del conejo se ha indicado que su abundancia junto a las carreteras puede ser uno de los factores asociados al atropello de turones (Mustela putorius) y a la proximidad del águila imperial ibérica (Aquila adalberti) a la carretera (Bautista et al. 2004, Barrientos and Bolonio 2009).

La disponibilidad de recursos como alimento o carroña atraen especies a las carreteras y pueden convertirlas en trampas ecológicas (Forman and Alexander 1998, Coffin 2007). Las especies de depredadores que se aproximen a los taludes se verán sometidas a un mayor riesgo de mortalidad por atropello. Generalmente, los depredadores ocurren a densidades menores que sus presas y poseen tasas de reproducción más bajas, por lo que la mortalidad por atropello tendrá mayores consecuencias en la población de depredadores que en la de sus presas (Fahrig and Rytwinski 2009). Si los depredadores son atraídos al tráfico y no son capaces de compensar dicha mortalidad a través de reproducción, las carreteras con altas densidades de conejos alrededor pueden convertirse en sumideros poblacionales para sus depredadores (Delibes et al. 2001).

1.3 La importancia de la escala, también en carreteras

En este último apartado me gustaría resaltar una de las cuestiones fundamentales en los estudios ecológicos, la escala. La escala a la que se observan los procesos ecológicos es determinante para los resultados obtenidos (Wiens 1989, Levin 1992). Los factores no afectan igual en todas las escalas y algunas respuestas son observables sólo a escalas concretas (Rettie and Messier 2000, Fortin and Dale 2005). Los estudios de los efectos de las carreteras suelen dar resultados diversos y a veces contradictorios. Así, la abundancia de grandes aves carroñeras sobre las carreteras parece ser mayores que en zonas alejadas cuando es estudiado a escala local, pero a escala de paisaje muestran preferencia por lugares alejados de las carreteras (Bautista et al. 2004, Lambertucci et al. 2009). Aunque estas diferencias pueden explicarse por características propias de las especies, el cambio de escala puede indicar la existencia de otros procesos a tener en cuenta. Algunos efectos son detectables sólo a nivel muy local, unos pocos de metros alrededor de las carreteras, mientras que otros requieren rangos espaciales más amplios. Por ejemplo, la existencia de presas en los taludes de las carreteras puede considerarse un atractor de depredadores. No obstante, el efecto atrayente dependerá de si la abundancia de presas en la matriz del paisaje en la misma, menor o mayor que la del talud. Asimismo, las respuestas de individuos observados junto a carreteras pueden corresponder con preferencias individuales y no de especie. Si este fuera el caso, hablaríamos de efectos locales de la infraestructura, mientras que si la respuesta se produce de forma generalizada y es detectable también a escalas amplias, podríamos hablar de un efecto de las infraestructuras sobre la especie. Por tanto, la escala a la que se realice el estudio puede afectar a las conclusiones obtenidas, lo

que probablemente ha generado la aparente contradicción en varios estudios de los efectos de carreteras.

1.4 Estructura y objetivos de la tesis

En primer lugar, me gustaría destacar que los objetivos de esta tesis se plantearon en el año 2009, cuando la mayoría de la literatura sobre los efectos de las carreteras en la fauna del entorno aún no existía. Se trata pues, de un trabajo novedoso que ha tenido que ir adaptándose al avance del conocimiento de forma paralela a su realización.

El principal objetivo de la presente tesis doctoral es aportar información que ayude a comprender mejor los efectos de las autopistas sobre las poblaciones de vertebrados de su entorno, centrándose en los aspectos de que la alteración del hábitat tiene en las poblaciones naturales a través de las relaciones depredador-presa. A partir de los análisis realizados se pretende proponer mejoras en las medidas de mitigación de los efectos negativos de las autopistas para minimizar sus consecuencias sobre la fauna.

Por su papel de presa clave en el ecosistema, se ha tomado el conejo como especie de referencia. La mayoría de estudios se centran en autopistas, por tratarse de construcciones con altos nivel de tráfico y límite de velocidad, que generan efectos más marcados sobre la fauna, y con taludes bien delimitados por vallas perimetrales.

La tesis se estructura en cinco capítulos centrales en formato artículo (Capítulos 2-6), seguidos de una discusión general (Capítulo 7) y conclusiones finales

(Capítulo 8). Los Capítulos 2 y 3 están dedicados al estudio de la respuesta en abundancia de una especie presa, el conejo europeo, a la proximidad de una autopista y sus características. En concreto, el Capítulo 2 se centra en las variaciones en abundancia en relación a la distancia a la autovía en un área de hábitat natural del conejo, y el Capítulo 3 explora cuáles son las características tanto del medio como de la propia infraestructura responsables de la abundancia de conejos en los márgenes de las autopistas.

Los dos siguientes capítulos analizan el uso del hábitat de los depredadores, aves rapaces y carnívoros, en función de la abundancia de presas y la proximidad a la autopista. El capítulo 4 explora la respuesta en el uso del hábitat de depredadores aéreos (rapaces diurnas), a la presencia de autopistas y carreteras en el entorno, teniendo en cuenta la disponibilidad de presa. Este estudio se realiza a escala de paisaje y posteriormente se comprueba si los patrones obtenidos son consistentes con datos del uso del hábitat en rapaces tomados sólo en el entorno próximo a las infraestructuras.

El Capítulo 5 se centra en depredadores terrestres, la comunidad de carnívoros medianos, y también investiga la respuesta de estos en dos escalas espaciales. A gran escala, se investiga si hay diferencias en la abundancia de carnívoros en zonas adyacentes a autopistas y zonas alejadas, y su respuesta a la abundancia de una presa principal, el conejo. Más en detalle, se analiza si la abundancia de conejo en los márgenes de autopista se relaciona con un aumento de los carnívoros y de sus atropellos.

El Capítulo 6 se centra en datos de atropellos tomados en el campo e investiga la asociación entre los atropellos de diversos taxones que cumplen el papel de presas en el ecosistema con los atropellos de sus depredadores, tanto a una escala

temporal como espacial.

Por último, en el Capítulo 7 se hace un síntesis de los resultados de todos los capítulos anteriores y una interpretación más amplia de ellos, seguido de una conclusiones finales presentadas en el Capítulo 8.

Chapter 2

Motorway verges: Paradise for prey species? A case study with the European rabbit

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Abstract

Roads have many effects on the mammal populations of their surroundings. Prey species are thought to establish dense populations in road verges due to a predation release effect, which arise as a side-effect of roadside avoidance by predators and/or predator roadkill. A species that has been suggested to benefit from predation release and attain high densities near roads is the European rabbit, a keystone species in Mediterranean ecosystems. We monitored rabbit relative abundance at three distances from a motorway (50, 450 and 850 m) during a 6 month period, as well as hunting and predator pressures, in a suitable area for rabbits. The lowest rabbit abundance was found next to the motorway (6.76 ± 8.87 pellets/m2 per month) and the highest abundance at an intermediate distance (17.65 ± 23.11 pellets/m2 per month). Hunting and carnivore pressures were highest at the sampling transect located farthest from the infrastructure. Thus, variability in rabbit abundance did not match the predation release effect found close to the motorway, and some sort of road avoidance or other process must underlie the observed abundance pattern. We advocate for a formal measurement of prey populations response to roads prior to any generalization as, in the case of rabbit, the response to roads and the potential cascading effects on other species may depend on landscape characteristics.

2.1 Introduction

Roads transform the environment, giving rise to multiple effects on the mammal populations of their surroundings (Trombulak and Frissell 2000, Fahrig and Rytwinski 2009). In most cases they have pernicious effects on mammals through fragmentation or mortality (Forman et al. 2003, Jaeger et al. 2005, Coffin 2007), but in some occasions they may have positive effects on species that make use of road verges as corridors or habitats. This seems to be the case of several small mammal species, which establish themselves in vegetated verges and can become abundant near roads (Bellamy et al. 2000, Brock and Kelt 2004, Sabino-Marques and Mira 2011). For example, Rytwinski and Fahrig (2007) found higher abundances of white-footed mouse (Peromyscus leucopus) in zones with higher road density, and another study carried out in USA found that juveniles of two lagomorph species (*Sylvilagus audubonii* and *Lepus californicus*) were only in areas adjacent to roads (Bissonette and Rosa 2009). Typical life history traits of small mammals, e.g. high reproductive rates, allow them to establish near roads, replacing very quickly the individuals lost to the population by traffic mortality (Rytwinski and Fahrig 2012). The presence of a steady and relatively abundant supply of prey alongside roads may attract predators and increase their likelihood of being run-over (Little et al. 2002, Ramp and Ben-Ami 2006) with two main consequences. From a biological perspective, the increased mortality of predators could benefit the populations of small mammals in areas of high road density due to the predation release effect (Rytwinski and Fahrig 2012). From the conservation and management viewpoints, it could be an issue of concern given that mammal predators usually have low population densities, low fecundity, and large home ranges, and all these characteristics make their populations particularly

vulnerable to increased mortality provoked by traffic (Spellerberg 1998, Trombulak and Frissell 2000, Forman et al. 2003).

Predation release has been proposed as a cause for the apparent high densities of wild European rabbits (*Oryctolagus cuniculus*) observed close to roads, together with the effects of lower hunting pressure and the presence of suitable soil to build warrens (Bautista et al. 2004, Barrientos and Bolonio 2009). However, very few studies have examined species abundance as a function of distance from roads, especially in lagomorphs, and results are somehow contradictory (see a review in Benítez-López et al. 2010). A North American study by Bissonette and Rosa (2009) found a greater abundance of two lagomorph species (*Sylvilagus audubonii* and *Lepus californicus*) alongside roads. However, a study focused in the European hare (*Lepus europaeus*), found greatest abundance at a distance of 400–500 m from motorways (Roedenbeck and Voser 2008).

The European rabbit is a key prey species in Mediterranean ecosystems (Delibes-Mateos et al. 2007), and the rabbit itself is currently of serious conservation concern since its populations in its natural range have declined by 70% in 30 years (Virgós et al. 2007). The rabbit also has a major impact on the ecosystem structure, influencing the floristic composition and giving rise to the heterogeneity on which many other organisms depend (Delibes-Mateos et al. 2008b, Gálvez-Bravo et al. 2009). In addition to this, the species is important from a social perspective, producing significant economic benefits as a game species (Fa et al. 1999) but also causing agricultural losses by feeding on crops (Martinez et al. 2003, Barrio et al. 2010).

Although some studies have related the presence of European rabbits with an increased road mortality of predators (Barrientos and Bolonio 2009, Barrien-

tos and de Dios Miranda 2012), no study has compared wild rabbit abundance near roads with that in the surrounding matrix. This information will help us evaluate rabbit response to roads, which can have important implications, as predator road mortality could be a by-product of high prey abundance. In that case, it should be a factor to consider when designing measures to minimize road impact on wildlife since several species of conservation concern, such as the Spanish imperial eagle (*Aquila adalberti*) and the Iberian lynx (*Lynx pardinus*) are among rabbit predators. In fact, both species are affected by roads and/or roadkills (Ferreras et al. 1992, Bautista et al. 2004).

The aim of this study is to estimate the variability in rabbit abundance related to distance from a motorway, as well as variations in hunting pressure and carnivore abundance at that scale as a measure of predation risk. This information will determine both whether there is an area of predation release near the motor-way, and whether rabbit abundance responds to it. We predict that rabbits will be more abundant closer to the motorway, where hunting and carnivore pressures will be lower.

2.2 Material and methods

2.2.1 Study area

The study was conducted in central Spain, adjacent to A-3 motorway, in Madrid province (40º12'N, 3º 19'W). The climate is continental Mediterranean, with a mean annual precipitation of 438 mm and a mean annual temperature of 13.8ºC. The substrate was similar throughout the area, comprised by a mixture of marls, clays and gypsum which are very suitable to warren building. Three

main natural vegetation formations could be distinguished throughout the area: steppe dominated by *Stipa tenacissima* on sunny slopes (*Stipa* formations), grassland dominated by gypsophilous plants on clay outcrops (Grasslands), and thyme (*Thymus* spp.) scrub elsewhere (*Thymus* formations). In addition, there were some small field crops, most of these being fallow during the study year, except for some olive and almond groves (Orchard crops). This mosaic landscape is typical from this part of Spain and is one of the most suitable for rabbits in Mediterranean ecosystems (Fa et al. 1999), being one of the areas with highest rabbit density in Spain (Villafuerte and Delibes-Mateos 2007). The four-lane A3 motorway was opened to traffic in 1995 and had a mean traffic volume of 31,462 vehicles per day in 2008 (Ministerio de Fomento-Gobierno de España 2010). At 5–10 m from the road there was a perimetral fence 1.5 m tall and made of wire mesh of 30.5 × 15 cm built according to 1990s road building regulations to prevent human and large animals to enter the motorway. Since it was neither reinforced nor pinned to the ground and the mesh spaces were large, it meant no obstacle to the movement of rabbits and carnivores.

2.2.2 Sampling design and data collection

In order to investigate changes in rabbit abundance, three strips (A, B and C) were marked out in the study area at increasing distances from the motorway, and they were surveyed for 6 months. Strip A began along the line of the motorway boundary fence and the other two strips ran parallel to it. Each strip was 2000 m long and 100 m wide, with an inter-strip separation of 300 m, being the mean distance between each strip and motorway of 50, 450 and 850 m, respectively. The separation between strips was chosen in relation to the average home range

size of a rabbit (circa 1 ha or smaller, Lombardi et al. 2007, Devillard et al. 2008), so that one home range would not extend over two strips. Moreover, the strip length allowed it to cross the home ranges of several independent rabbit groups.

We randomly selected 20 points inside each strip ('HawthsTools' extension, ArcGis 9.2, ESRI, 2009). We estimated an index of rabbit abundance by pellet counts in pellet clearance permanent plots (Palomares 2001). This method has been validated recently as more reliable in relation to rabbit abundance than other abundance indices for the Iberian Peninsula, such as the number of burrows or latrine counts (Fernandez-De-Simon et al. 2011a). The fieldwork took place from March to September 2009 and each plot was surveyed monthly. In March, one permanent plot of 0.6 m of radius (1.13 m2) was placed at each point, and all the rabbit pellets were cleared. Plots were placed avoiding unsuitable areas, such as orchard crops, steep slopes and rabbit latrines, in which the accumulation of pellets could not reflect the abundance of rabbits. From the first visit on, once a month we counted the accumulated pellets inside each circular plot and cleared them for the next visit.

Since pellet persistence can differ between sites (Iborra and Lumaret 1997, Fernandez-de-Simon et al. 2011b), we estimated a rate of pellet decay in each strip. Four control plots were evenly spaced along each strip, protected by a wire mesh that excluded rabbits, but not rain or coprophagous beetles. We placed 30–40 fresh pellets in each plot every visit and counted those remaining in the next month, before clearing and replacing them for new fresh pellets. Accordingly to other studies that found that one month time is short enough for disintegration rates not to affect the study results (Moreno and Villafuerte 1995), we found very high permanence rates (93.5 ± 1.34% pellets) and similar along the whole study

area. Therefore, we did not use decay rates to correct the values of the abundance index.

Parallel to the rabbit abundance measures, we also estimated indices of carnivore pressure and hunting pressure within the strips. One transect of 2 km long and 2 m wide was walked in each strip once a month by the same observer. The count of carnivore scats in the transect was used as an index of carnivore strip-use intensity, and thus of relative potential predation pres-sure in it. Assuming that carnivores mark more frequently those parts of the study area more visited, the number of scats in a strip should be related to the time that a carnivore spends there and with an increased risk of predation for rabbits. We cleared the transect lines of carnivore scats in March and collected data from April to September, clearing all the scats found in each visit. All the material we found were originated from mid-sized carnivores: cats (*Felis* sp.), foxes (*Vulpes vulpes*) and mustelids (*Meles meles* and *Martes foina*), all of which feed on rabbits in Mediterranean ecosystems (Delibes-Mateos et al. 2008b), so we opted to analyze all the data together as "carnivores". The index of hunting pressure was defined as the number of spent cartridges found in each transect. The hunting season ends in February and does not recommence until September, so we walked slightly different transects every month and those cartridges found in March were also included in the analysis. The cartridges were removed in each visit to avoid possible double counting where transects overlapped.

Differences in vegetation affect rabbit abundance at different spatial scales (Fa et al. 1999, Virgós et al. 2003). Our study area was not big enough to present differences in macrohabitat characteristics, but differences in microhabitat around the sampling plots were possible. Thus, we estimated the percentage cover

of vegetation types within 25 m around each plot (Virgós et al. 2003), in order to compare the vegetation between strips and to evaluate possible microhabitat effects on our results. We defined five main vegetation types in the study area (see study area description for more details): Grasslands, *Thymus* formations, *Stipa* formations, Orchard crops (olive and almond) and others (rocks, paths, bare ground). Vegetation cover was estimated by analyses of aerial photographs with ArcGis 9.2 (ESRI, 2006).

2.2.3 Data analyses

Differences in rabbit abundance were analyzed using a general linear mixed effects (LME) model that enables the analysis of longitudinal data taken in the same experimental unit along time. The correlation between explanatory variables was tested prior to model building to avoid collinearity. There was no coefficient of correlation above 0.7, so all the variables were introduced in the full model. Model building and evaluation was done following Zuur et al. (2009). Plot identity was used as the only random factor, since the inclusion of other variables into the random part did not improve the explicative power of the model according to the likelihood ratio test (LRT). The fixed part of the model included the categorical variables month and strip and the continuous variables of vegetation types. Also the interaction between strip and month was included, in order to test if rabbit abundance differently changed in each strip along the sampling period. In this case, we looked for a steeper decline in rabbit abundance in Strip A that would reflect the extra mortality caused by the motorway.

Regarding vegetation characteristics, only four vegetation variables were used as explicative variables, given that the cover of the five vegetation types es-

timated by this method is constrained to 100% and, thus, the information of the fifth variable is included in the other four. These variables were transformed using the arcsin transformation for proportions. Also, the response variable, rabbit abundance index, was log-transformed. The assumptions of the model were checked in the residual plots. Following suggestions of Forstmeier and Schielzeth (2011) on avoiding multiple hypotheses testing in linear models, we present the full model in the Results section.

As detecting differences in rabbit abundance between strips were our main objective, differences between pairs of strips were tested by a post hoc Tukey HSD test. Additionally, differences in vegetation among strips in microhabitat surrounding the plots were tested by one-way ANOVA to evaluate the homogeneity of the study area.

We analyzed relative carnivore and hunting pressures by χ^2 test, the null hypothesis being a uniform distribution among samp-ling strips of both carnivore faeces and spent cartridges. Tests were performed by 10,000 randomizations using EcoSim 7.72 software (Gotelli and Entsminger 2009).

Other statistical analyses were performed with STATISTICA 8.07 (StatSoft, Inc., 2007) and R v. 2.15.0 (R Core Team 2012), using packages nlme (Pinheiro et al. 2012) and multcomp (Hothorn et al. 2008).

2.3 Results

We found a total of 917 pellets in Strip A, 2344 pellets in Strip B and 1908 pellets in Strip C. Mean pellet counts (±SD) were 6.76 ± 8.87 pellets/m2 per month

in Strip A, 17.65 ± 23.11 pellets/m2 per month in Strip B, and 14.22 ± 22.23 pellets/m2 per month in Strip C. In the LME model to explain rabbit abundance only the predictors Strip and Month were significant (Table 2.1). None of the vegetation variables had a significant effect on the number of rabbit pellets per plot ($p > 0.05$ in all cases). Also the interaction between Month and Strip was not significant.

We found a steady reduction in rabbit abundance along the sampling period and significant differences in abundance between Strips A and B (Tukey HSD, $p < 0.05$) (Fig. 2.1). Strip B had the highest rabbit abundance in the study area, whereas Strip A had the lowest. Strip C had an intermediate rabbit density that did not differ significatively from that of the other strips ($p > 0.05$).

Table 2.1. Results of the linear mixed model for rabbit abundance index. The model included plot identity as random factor.

Predictors	**Df**	**F value**	**p**
Intercept	282	207.94	<0.001**
Month	282	5.48	<0.001**
Strip	53	3.29	0.045*
Grasslands	53	0.50	0.481
Thymus formations	53	0.94	0.337
Stipa formations	53	0.80	0.375
Orchard crops	53	1.97	0.166
Strip × month	282	1.37	0.192

* $p < 0.05$; ** $p < 0.01$

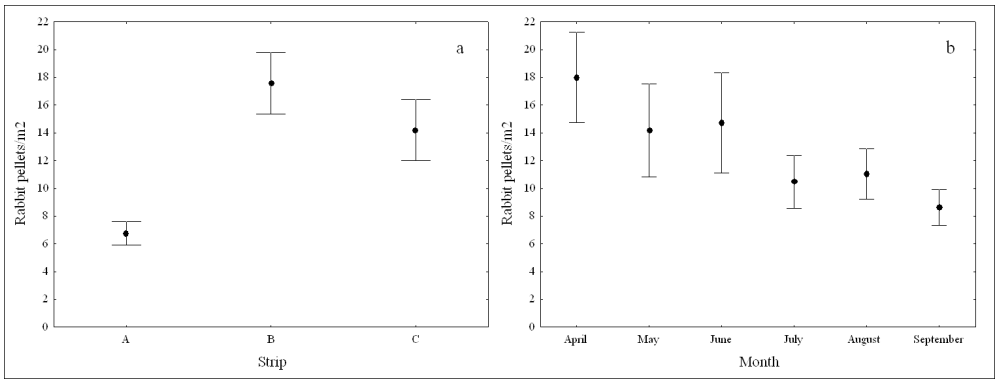

Figure 2.1. Number of rabbit pellets/m^2 (mean ± SE) found in each strip (a) and each month (b).

Cover percentage of each vegetation type can be found in Table 2.2. One-way ANOVA of vegetation cover showed no significant differences among strips in cover of any vegetation type ($p > 0.05$ in all cases), thus confirming the homogeneity of the study area.

Table 2.2. Mean values (±SDs) of the cover percentage of each vegetation type per strip and in the whole area.

	Cover percentage			
	Strip A	**Strip B**	**Strip C**	**Total**
Grassland	15.75 ± 17.79	13.70 ± 16.95	8.30 ± 10.10	12.58 ± 15.41
Thymus formations	34.20 ± 25.40	50.80 ± 27.62	44.48 ± 28.36	43.16 ± 27.57
Stipa formations	27.52 ± 28.95	18.42 ± 27.10	36.08 ± 34.95	27.34 ± 30.86
Orchard crops	15.74 ± 30.92	14.98 ± 28.74	6.71 ± 17.83	12.48 ± 26.33
Others	7.95 ± 11.66	2.14 ± 5.80	4.67 ± 9.16	4.92 ± 9.35

The values of carnivore and hunting pressures indices are shown in Table 2.3. The carnivore pressure index showed significant differences between Strips A and C (χ^2 = 11.62; p = 0.047), and marginally significant differences between Strips B and C (χ^2 = 11.38; p = 0.051), with no significant differences between Strips A and B (χ^2 = 4.76; p = 0.487), being the highest values from the strip furthest from the motorway. The hunting pres-sure index showed significant differences between Strip C and the other two (B–C, χ^2 = 17.52; p = 0.013; A–C, χ^2 = 24.09; p < 0.001), and marginally significant differences between Strips A and B (χ^2 = 12.11; p = 0.098), the highest values once again being from the strip furthest from the motorway.

Table 2.3. Values obtained for the predator pressure index (number of carnivore scats per transect) and hunting pressure index (number of cartridge cases per transect).

	Predator pressure		**Hunting pressure**	
	n	**Mean ± SD**	**n**	**Mean ± SD**
Strip A (0-100 m)	6	1.67 ± 1.86	7	11.71 ± 15.42
Strip B (400-500 m)	6	2.17 ± 2.32	7	11.43 ± 10.53
Strip C (800-900 m)	6	3.00 ± 1.67	7	15.00 ± 17.50

2.4 Discussion

The results do not support the prediction that rabbit abundance is greatest next to the motorway. On the contrary, the area nearest to the motorway showed the lowest rabbit abundance in our study area. However, we did find the existence of a refuge from hunting and carnivores near the motorway, i.e. the expected predation release effect for rabbits (Bautista et al. 2004), but it did not lead to

an increased abundance of the species, thus it seems not to act as a real refuge. These results point to the possibility that small mammal concentration in road verges could not be a general response, at least for European rabbits, and further research is needed to establish the relationship between rabbit abundance and roads. We suggest a more detailed study of this subject in other cases when rabbit abundance has been assumed to be higher next to road without checking it.

Our study showed that the highest rabbit densities were at an intermediate distance from the motorway (Strip B). A similar result has been obtained for the European hare in Switzerland (Roedenbeck and Voser 2008). The low abundance could be due to several factors, like dispersal of the juveniles or high mortality by traffic or predators. One potential explanation could be that populations close to the road remain low because of roadkill. However, our six-month monitoring of rabbits showed that the decline in abundance along the study period was similar in all three sampling strips (no significant effect of the interaction month × strip, Table 1). Thus, there is not a significant additional mortality in populations in Strip A such as the one expected if the motorway was responsible for more rabbit deaths than other mortality factors in the area. Also, movement of rabbits between strips, not measured in this study, could explain to some degree our results, though its intensity should just compensate numbers so that patterns of change are similar among bands.

Carnivore pressure tended to decline near the motorway, as it was hypothesized from the outset. This finding is consistent with previous studies that found roads to have a negative effect on carnivores (Fahrig and Rytwinski 2009, Benítez-López et al. 2010). However, the reduced presence of carnivores did not lead to the expected increase in the population of the prey, as it has been detec-

ted for other species (Rytwinski and Fahrig 2007). This is a noteworthy finding as mesocarnivore abundance is known to act as a powerful regulator of rabbit population densities under very different settings, especially when rabbit populations are not in high densities (Pech et al. 1992, Palomares et al. 1995, Banks 2000). In our study, we found higher abundance of rabbit in zones with more carnivores. Regarding the case of the European rabbit it is important to remember that former studies did not analyze animal densities in the proximity to roads in comparison with control sites (Barrientos and Bolonio 2009, Barrientos and de Dios Miranda 2012). Also, we found the proximity of the motorway to be a partial refuge from hunting, with significantly higher hunting pressure in the furthest strip. The fact that the number of cartridge cases in Strips A and B was similar (and lower) should be interpreted from the human perspective: hunters somewhat reduce their activity close to the road due to the legal ban, though some spent cartridges can be found barely ten metres from the motorway fence. As a result, hunting pressure does not fit rabbit abundance at the spatial scale of the study. The potential effect of avian predators remains to be known, though it is usually of lesser intensity (Lombardi et al. 2003). In sum, in the study area a 'global predation release effect', both from terrestrial carnivores and from hunters, occurs near the motorway.

The microhabitat present close to the road, a potentially confounding factor, was not responsible for the detected pattern of rabbit abundance. Our results did not show any microhabitat effect on rabbit abundance nor habitat differences among sampling strips. Vegetation type has been reported in the Iberian Peninsula to determine rabbit abundance at a landscape scale (Virgós et al. 2003, Fernández 2005), and land use stands out among the factors that influence rabbit abundance at larger scales (Lombardi et al. 2007). However, such kind of effects

should be acting at larger spatial scales, like those referred below in relation with regions devoted to agriculture.

Rabbits could avoid at some degree the area near the motorway due to a combination of several factors, and experimental data would be needed to discern among possibilities (Fahrig and Rytwinski 2009). Noise or other traffic annoyances could affect rabbit populations near roads, maybe causing stress to the individuals (Monclús et al. 2009, Navarro-Castilla et al. 2011). Under such circumstances, rabbits could establish in road verges but their populations would not reach very high numbers (Coffin 2007). Our results seem to indicate that the side-effects of traffic could be more powerful than predation in regulating rabbit populations, at least, in our study area.

The detected pattern in rabbit abundance may not occur every-where and relatively high rabbit densities may occur in motorway verges where they are the only available habitat. Within agricultural regions with extensive crop cover, small mammals tend to settle in uncultivated patches (Bellamy et al. 2000). This could be especially apparent in the case of rabbits, given that periodical ploughing destroys their burrows and prevents them from establishing (Calvete et al. 2004). In such areas, rabbit populations should settle in uncultivated patches like road verges, and feed on fieldcrops (Barrio et al. 2010). Nevertheless, where the rabbit distribution is not restricted by habitat modification, as it was the case in our study area, the population near a motorway could have a marginal character, road verges not being selected preferently.

Finally and within the limitations imposed by the spatial, temporal and taxonomical limitation of our study, two main conclusions can be raised. First, it is important to advocate for a formal measurement of population responses to

roads prior to raising any generalization about the influence of such infrastructures on their abundance. In our opinion, the European rabbit should not to be viewed as a species with dense populations in road verges every-where and further research is needed. High densities may happen in agricultural regions or, at a finer scale, where structures associated to motorways provide a resource unavailable in the surroundings and valued by the species, e.g. easy to dig substrate in bridges (Rogers and Myers 1979), but not in other areas. Secondly, in case that rabbit populations settled beside motorways are responsible for cascading effects on other species, particularly carnivores, such effects should be expected to occur mainly in areas where rabbits do show higher densities in road verges than in the rest of the landscape. And even more if such situation coincides with well preserved areas rich in fauna at a regional scale or with the presence of corridors (Forman et al. 2003, Grilo et al. 2009).

2.5 Acknowledgements

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Chapter 3

Infrastructure features drive rabbit density around motorways

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Abstract

1. Human disturbance is widespread in the landscape in the form of roads that alter wildlife populations. Knowing which road features are responsible for the species response and their importance related to environmental variables will provide useful information for effective conservation measures.

2. We studied relative abundance of European rabbits, a very widespread species, in motorway verges in an area with a large variability in habitat and verge characteristics. We identified the most important variables related to the infrastructure or the environment by model averaging and AICc, and also performed a variance partitioning analysis to determine the relative importance of each group of variables.

3. Among environmental variables, the most important ones for rabbit abundance were related to the main habitat requirements of rabbits (low altitude, less productive sites, low tree cover). Within infrastructure features, the most important variables were traffic load and verge width, with negative and positive effects, respectively.

4. As a group, infrastructure features explained four times more variability in rabbit abundance than environmental variables, and motorways located in altered landscapes with no available habitat for rabbits, such as agricultural fields, had the largest effect on rabbit abundance in verges.

5. Given the role of rabbits in many ecosystems, from key prey to invasive species, we propose that motorway verges are kept unattractive to rabbits to avoid cascading effects on their predators by roadkill or the spread of the species in unwanted areas.

6.'Synthesis and applications'. The response of species to the studied human infrastructures can be modulated through the modification of motorway features, being some of them easily modified in the design phase. By identifying such features, we provide suggestions to improve conservation plans that include less expensive measures. As a general indication, keeping motorway verges less than 10 m wide and with non-palatable vegetation will prevent high densities of rabbits and avoid the unwanted effects that rabbit populations can generate in some areas.

3.1 Introduction

As human population and pressure on nature grow, many conservation efforts are devoted to minimize the effects of human disturbance, and for such a goal it is important to identify the main features responsible for wildlife populations' increase or decline in disturbed landscapes. Terrestrial ecosystems are increasingly altered by anthropogenic activities, affecting to some degree more than 80% of the land surface (Sanderson et al. 2002, Foley et al. 2005, Leu et al. 2008). Human activity modifies the interactions between the different elements of the ecosystems and the conditions under which the wildlife populations develop (Hobbs et al. 2006, Bozek et al. 2007, Clavel et al. 2011). To better understand wildlife responses and to improve environmental integration of human activities, those factors that contribute the most to population changes should be identified. Only then it will be possible to adopt effective conservation measures.

An outstanding case of human disturbance that is widespread and affect many different ecosystems is road infrastructure. Roads are well known drivers of ecosystem change (Trombulak and Frissell 2000, Forman et al. 2003), since they spread in the landscape and affect huge areas. For example, in the US at least the 22% of the land area is less than 100 m from a road and 80% is within 1 km (Forman 2000, Riitters and Wickham 2003). Among many others, one of the effects of roads is the creation of new habitat and corridors that are used by small mammals (Bennett 1990, Ruiz-Capillas et al. 2013b, de Redon et al. 2015). However, this effect may not be desirable in every situation, as it can act as corridor for invasive species (Downes et al. 1997, Trombulak and Frissell 2000) and also, it has been proposed that dense populations of prey near roads can attract predators and increase their mortality risk (May and Norton 1996, Barrientos

and Bolonio 2009, Grilo et al. 2014).

In order to minimize alterations in the landscapes, we should identify the key features that are responsible for wildlife populations' response to both the environment and the disturbance itself (the road in our case). Only then we can propose effective conservation measures as well as optimize the cost of mitigation actions, as several studies have emphasized (Malo et al. 2004b, van der Grift et al. 2013, Ward et al. 2015).

It is to be expected that wildlife populations in road verges respond to road features and habitat characteristics (Clevenger et al. 2003, Farmer and Brooks 2012, Santos et al. 2013). By identifying such features, we can anticipate which actions are expected to have more repercussion on the species and to give specific recommendations to managers and policy makers to maintain or avoid wild populations and pursue conservation. For example, managing verges to regulate prey populations may be a solution worth in economic terms to reduce the costs of installing and maintaining complex measures to avoid predator mortality or the spread of invasive species.

The European rabbit (*Oryctolagus cuniculus*) is a key prey species in Mediterranean ecosystems and classified as 'vulnerable' in Spain (Villafuerte and Delibes-Mateos 2007, Delibes-Mateos et al. 2008b, Gálvez-Bravo et al. 2011). Within its native range, rabbits are the preferred prey for most of the Iberian predators and therefore, a species of conservation concern (Virgós et al. 2007, Delibes-Mateos et al. 2008b). Meanwhile, in other areas of the world rabbits are invasive species that spread quickly in new environments, causing severe damage to the ecosystems they invade (Lees and Bell 2008). In addition, rabbits have shown a high tolerance to human disturbance and are among usual urban or rural

fauna (Garden et al. 2006, Baker and Harris 2007, Ziege et al. 2015). This species has the ability to use roadside verges as habitats, where they use embankments to build their warrens, as well as corridors to spread through the landscape (May and Norton 1996, Bautista et al. 2004, Planillo and Malo 2013). These characteristics make the European rabbit the optimal species for this study due to their implications in human altered landscapes: conservation problems in native range, plague or invasive species worldwide, as well as damages to infrastructures due to their digging activity.

In this study we analyze variability in rabbit abundance in the human altered habitat of motorway verges within its native range, with three main objectives: 1. Determine which features are correlated with rabbit abundance in motorway verges; 2. Assess the importance of the factors related to the disturbance (infrastructure and traffic) in relation to those of the wider environment for rabbit abundance; 3. Propose measures to manage rabbit abundance in verges. From our results, we make specific proposals for wild population management that could be cost effective, based on the analysis of the infrastructure features that better explain species abundance.

3.2 Methods

3.2.1 Study Area

We conducted our study in Central Spain, provinces of Ávila and Segovia (40°46'N 4°25'W), in an area of around 3000 km2 (55 x 55 km) which includes in wide range of environmental conditions (see supplementary Table S3.1). This region has a continental Mediterranean climate, with cold winters and an average

rainfall of 450 mm a year, mainly in autumn and spring. The study area incorporates a gradient of environmental conditions. The southern part is dominated by oak forests and pastures with oaks managed for cattle ("dehesas"), followed by a mixed landscape with small crop fields and patches of natural vegetation, and intensive non-irrigated crop fields in the most northern part. Three motorways can be found in the study area, which main characteristics are summarized in table 3.1.

3.2.2 Survey design and data collection

In spring 2010, we generated 100 random points –20 for each motorway section in table 3.1–, and we estimated in them an index of relative rabbit abundance. We did the field surveys in the shortest period of time possible, in order to avoid differences in the abundance of rabbits between motorways due to reproduction or mortality. Random points were separated a minimum distance of 300 m so that s single rabbit territory does not overlap two sampling points (home range 1 ha, Lombardi et al. 2007), and they were used as the starting point for the transects to estimate rabbit relative abundance in each motorway stretch. In our study area, landscape is divided into small private fields, making difficult to use long transects, therefore we opted to use the method described in Fa et al. (1999). This method consist in transects of 50 x 2 m where relative rabbit abundance is estimated by counting of pellet groups divided in five classes: 1: 1-2 pellets, 2: 3-15 pellets, 3: 16-50 pellets, 4: 51-150 pellets and 5: more than 150 pellets. Then, we multiplied the number of groups of each class by the mean number of pellets in that class and sum all the pellets to obtain the relative rabbit abundance in each transect.

Table 3.1. Description of the motorways in the study area, including the predominant vegetation (Main vegetation) and the characteristics of the perimeter fence around each one.

Motorway	Lengh	Route	Lanes	Traffic1	Main Vegetation	Perimeter fence
AP-6	27 km	Villacastín - Adanero	4	18,866	Small patches of natural vegetation and crop fields.	2 m tall progressive fence of wired mesh of 15x5 to 15x20 cm, without reinforcement in the lower part.
AP-6	20 km	San Rafael - Villacastín	4	29,155	Natural vegetation and grasslands.	2 m tall progressive fence of wired mesh of 15x5 to 15x20 cm, without reinforcement in the lower part.
A-6	30 km	Adanero - Ataquines	4	31,284	Intensive crop fields.	1.5 m tall progressive fence of wired mesh of 15x5 to 15x20 cm, not pinned to the ground.
AP-61	28 km	San Rafael - Segovia	4 – 6	6,294	Oak forest and dehesas.	2 m tall progressive fence of wired mesh of 15x5 to 15x20 cm, with a reinforcement in the lower part of 5x5 cm and pinned to the ground.
AP-51	25 km	Villacastín - Ávila	4 – 6	8,450	Dehesas and crop fields.	2 m tall progressive fence of wired mesh of 15x5 to 15x20 cm, with a reinforcement in the lower part of 5x5 cm and pinned to the ground.

[1] Average daily traffic (ADT) in 2009.

To properly characterize the rabbit abundance in each motorway stretch, we designed a system of four transects at each sampling point. Two transects on each side of the motorway, one outside the perimeter fence and the other one inside the fence (Fig. 3.1), and we estimated a relative rabbit abundance for each one.

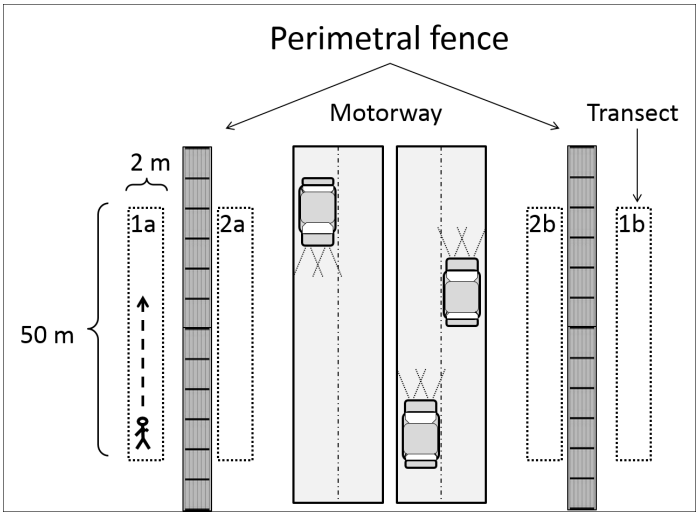

Figure 3.1. Survey design for estimating the rabbit abundance index. Detail of each highway stretch with four parallel transects, two outside the perimeter fence (transects 1a and 1b), and two inside (transects 2a and 2b).

Rabbit abundance in motorway verges may respond to characteristics of the landscape or of the motorway, and thus we divided explanatory variables into variables related to the environment (environmental variables hereafter), and variables related to the infrastructure (infrastructure variables hereafter). Environmental variables are descriptive of the landscape and may be seen as the underl-

ying natural variables that shape the distribution and abundance of the species, usually out of management capabilities of road managers. On the contrary, infrastructure variables depend on the design of the motorways and some of them can be modified in response to management objectives.

A. Environmental variables

We measured the main habitat characteristics in the environment surrounding the motorway that could affect rabbit abundance (Supplementary Table S3.1): altitude ("altitude"), vegetation cover (simplified by PCA, see below), vegetation productivity measured by NDVI ("ndvi"), distance to closest river ("dist.water"), and soil hardness. We used altitude as a proxy for climate, as in our study area it is negatively related to temperature and positively related to rainfall, both factors affecting rabbit populations (Palomares 2003, Calvete et al. 2004). We characterized the vegetation in 50 m plots around each transect by two variables: the cover percentage of vegetation types (herbaceous, shrubs, trees, crops, unproductive), and the productivity of the vegetation measured by the Normalized Difference Vegetation Index (NDVI). Both variables were estimated by aerial images in a public repository of Castilla y León Government (Junta de Castilla y León 2011). Rivers are used as corridors by many species (Virgós 2001, Matos et al. 2009, Sabino-Marques and Mira 2011), thus wildlife species will have easiser access to areas near them. Finally, soil hardness may limit the capacity of rabbits to dig warrens, but we found very low variability in soil hardness in our study area. Therefore we decided not to include this variable in the analyses.

B. Infrastructure variables

We estimated five variables describing the motorway and the conditions in the verges that may affect rabbit abundance (Supplementary Table S1): traffic volume ("adt"), the width of the verges ("verge.width"), the presence of embankment ("embank"), and the cover of different types of vegetation within the fenced area (verges, see below): herbaceous, shrubs, trees, and unproductive. The traffic volume has been related to disturbance and mortality (Clarke et al. 1998, Forman et al. 2003, Benítez-López et al. 2010), and it was provided as average daily traffic (ADT) by the company responsible for the motorways (Iberpistas S.A.). We estimated the cover percentage of the vegetation classes in the whole verge parallel to each transect using aerial photographs. As these variables were correlated, to avoid collinearity problems in the models we decided to use only the cover of herbaceous plants ("cover.herb"), as an indicator of food availability for rabbits. The width of the verges is a measure of the amount of habitat provided by the infrastructure and was defined as the distance from the asphalt surface to the perimeter fence. We took the distance in meters in the center of each sampling transect. Finally, we evaluated the presence of embankment ("embank") if the road was over the ground level at least in one side of the motorway, as the embankments provide potential ground for warren building in an elevated that prevents flooding during rain periods.

3.2.3 Data analysis

As a first approximation, we contrasted the data obtained from transects outside and inside the perimeter fence (Fig. 3.1) to test whether the fence had an effect on rabbit abundance. We did a Spearman correlation and obtained a value of

rho = 0.91, $p < 0.001$ (n = 98). We concluded that perimeter fences were not an obstacle for rabbits and relative abundances measured outside were representative of rabbit abundance in the verges and we use data from the outside transects in further analyses, for simplicity.

To characterize each motorway stretch, we computed the mean value of all variables measured in both sides. The cover percentages of vegetation types in the landscape outside the fence were simplified by a PCA to reduce the number of variables in the analyses and we used the first two axes as a summary of vegetation (Supplementary information, Fig. 3.4). Axis 1 distinguished between the abundance of crops (positive values) and cover of herbs and shrubs (negative values). Axis 2 represented a gradient between unproductive areas (positive values) and high tree cover (negative values).

We tested the correlation between explanatory variables and no correlation higher than 0.7 was found, thus all variables were included in the analyses. The linearity of the relationship between explanatory variables and rabbit abundance was inspected by univariate plots and explanatory variables were transformed when necessary. Distances were log-transformed and proportions were transformed by arcsin. Also, traffic volume suggested a quadratic relationship and thus, it was introduced in the model in the quadratic form. The response variable was transformed by square root transformation to meet the normality criterion.

We tested for spatial autocorrelation in rabbit abundance by Moran correlogram and found positive correlation. To account for this, we used Generalized Least Squares (GLS) regression models with spherical structure for spatial autocorrelation based on the semivariogram values (Dormann et al. 2007, Beale et al. 2010). We run a full GLS model with all the explanatory variables and used

model selection by Akaike Information Criterion corrected for small sample size (AICc) to obtain the best models (Burnham and Anderson 2002). We consider to be competitive all models within two points of AICc. Then we computed the averaged model and obtained the averaged coefficients for the selected explanatory variables. All explanatory variables were standardized to make the coefficients comparable (Quinn and Keough 2002). Model residuals were checked for model assumptions.

Finally, we analyzed the explanatory power of each group of variables, using variance partitioning analysis (Borcard et al. 1992, Ferrer-Castán and Vetaas 2005, Peres-Neto et al. 2006). For this analysis we used three groups of variables: environmental, infrastructure and spatial variables. For the environmental and infrastructure groups we included only those variables selected in the GLS averaged model. Spatial information was first tested on the response variable using a cubic regression and simplified using backward selection. Only the spatial variables remaining were used in the variance partition analysis (Borcard et al. 1992). All analyses were done in R 3.1.1 (R Core Team 2014), using libraries nlme for GLS models (Pinheiro et al. 2014), MuMIn for model averaging (Barton 2014), and vegan for variance partitioning (Oksanen et al. 2015).

3.3 Results

We collected data of rabbit relative abundance in both sides of the motorway in 99 out of 100 sampling points, with values of the index ranging from 0 to 4662. As transects were 50 x 2 m, this means that some transects had a density of 40 pellets/m2. Obtained values covered a wide spectrum of abundances, from absence

to high rabbit abundance and intermediate situations (Fig. 3.2).

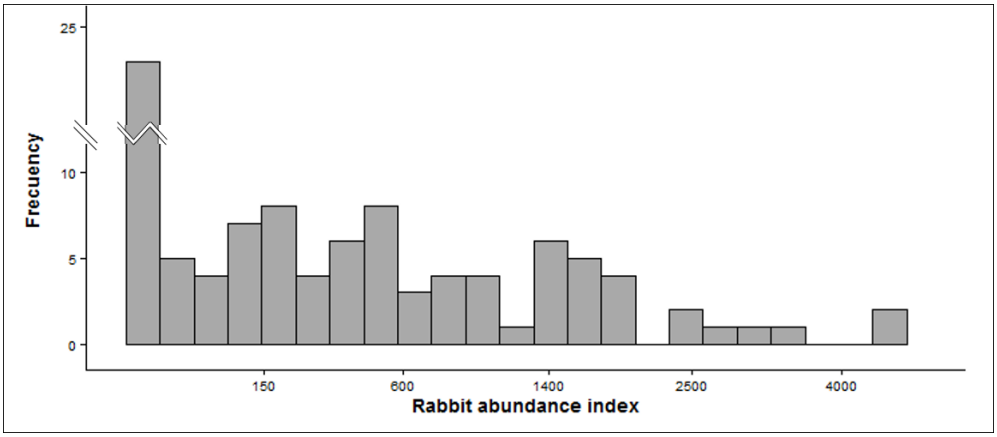

Figure 3.2. Frequencies of rabbit abundance index found in the 99 motorway stretches surveyed. The vertical axis is discontinued.

The model selection process of the GLS for rabbit abundance returned 11 models within two points of AICc (Table 3.2). The variables selected and their averaged coefficients are shown in Table 3.3. Within the environmental variables, the most important were the altitude and the productivity of vegetation, as measured by NDVI, followed by vegetation cover represented by the axis 2 of the PCA. The coefficients of the averaged model showed that rabbit abundance was higher at places with lower altitude - less rainfall and moderate temperatures in winter-, low productivity as measured by NDVI values, and positive values of axis 2, pointing to open areas with low tree cover. Amongst infrastructure variables, two of them were retained in all models, thus being the most important variables for explaining rabbit abundance: the width of the verge and the traffic volume. The width of the verge had a positive effect, with increasing rabbit abundance in wider verges. The effect of traffic was negative and increasingly so for higher

Table 3.2. Model selection table with all models selected within two points of AICc. Explanatory variables are grouped as infrastructure or environmental variables.

Environmental variables				Infrastructure variables				R2	df	AICc	ΔAICc	Weight
altitude	ndvi	axis2	axis1	verge.width	adt	adt2	herb.cover					
-0.307	-0.126	0.102		0.202	-0.005	-1.046		0.63	8	199.1	0	0.156
-0.295	-0.113			0.219	-0.008	-1.066		0.62	7	199.5	0.35	0.132
				0.184	0.098	-1.082		0.6	5	200	0.9	0.1
-0.234				0.220	0.015	-1.037		0.61	6	200.2	1.05	0.092
	-0.099	0.096		0.158	0.104	-1.097		0.62	7	200.4	1.29	0.082
	-0.088			0.176	0.097	-1.114		0.61	6	200.4	1.32	0.081
		0.085		0.169	0.105	-1.064		0.61	6	200.4	1.33	0.081
-0.239		0.088		0.205	0.020	-1.017		0.62	7	200.5	1.39	0.078
-0.357	-0.131	0.099		0.201	-0.040	-0.930	0.103	0.63	9	200.6	1.53	0.073
-0.349	-0.119			0.217	-0.045	-0.940	0.112	0.62	8	200.8	1.7	0.067
-0.330	-0.136	0.100	-0.062	0.180	-0.001	-1.078		0.63	9	201.1	1.96	0.059

Table 3.3. Variables included in the selected models within 2 points of AICc, with the number of models in which they were present out of 11 best models, the Akaike weight of the variable and their coefficient in the averaged model.

Variable	Models	Σwi	averaged coefficients ± SE
Environmental			
altitude	7	0.66	-0.20 ± 0.20
ndvi	7	0.65	-0.08 ± 0.08
axis2	6	0.53	0.05 ± 0.07
axis1	1	0.06	-0.01 ± 0.03
dist.water	0	-	-
Infrastructure			
verge.width	11	1	0.20 ± 0.09 *
adt	11	1	0.03 ± 0.15
adt2	11	1	-1.05 ± 0.25 ***
herb.cover	2	0.14	0.01 ± 0.06
embank [Presence]	0	-	-

* Significant at 0.05; *** Significant at the 0.001.

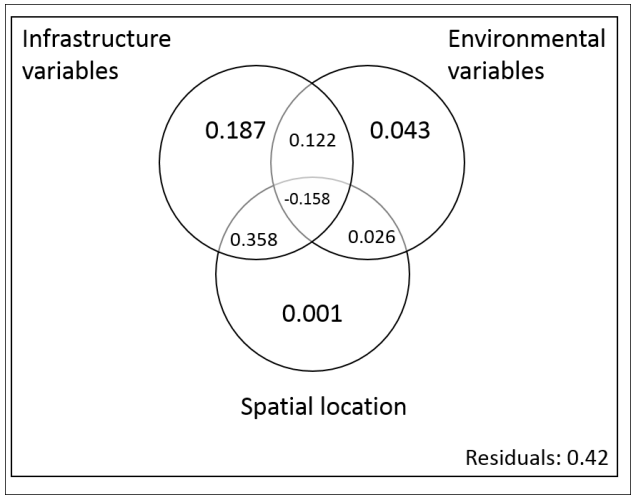

Figure 3.3. Proportion of variance explained by each group of variables (big number in circles) and shared portions (numbers in the overlapped regions).

traffic volumes, as shown by the quadratic term.

In the variance partitioning analysis (Fig. 3.3), the environmental variables were those with less explanatory power, accounting only for 3% of the total variance (when taking into account the shared fractions). In contrast, the group of infrastructure variables explained 50.9% of the variance in rabbit abundance. The spatial location was the second most informative group, explaining 22.7% of variance, although most of it was shared with the infrastructure variables, as the infrastructure is inherently spatially continuous. Looking at the pure effect of the groups -without the shared fraction-, infrastructure variables explained four times the variance explained by environmental ones (18.7% and 4.3%, respectively).

3.4 Discussion

Our assessment of the importance of different variables for explaining rabbit abundance in the area surrounding motorways found that those variables related to the infrastructure had a heavier weight than those of the environment. Environment poses a large scale frame where the species can survive. However, the variability in rabbit abundance at a small scale is better explained by the modifications to the habitat generate by the motorway. Human disturbance is present in many landscapes (Sanderson et al. 2002, Foley et al. 2005) and it combines with natural conditions to shape wildlife populations. Thus, the response of rabbits to the infrastructure could be modulated by managing key motorway features, while assuming the presence of effects associated to unmanageable variables, such as traffic volume or climatic conditions.

The environmental variables selected in the best models are those related

with the distribution of the species at a broad scale. Altitude was the most important one, as European rabbit is a species related to Mediterranean climate, and more Mediterranean conditions occur at places with low altitude in our study area. At higher altitutes, heavy rains or too cold climate in winter may limit the population (Palomares 2003, Calvete et al. 2004). The other two important environmental variables (NDVI, axis2), were related to the vegetation and both pointed to rabbit flourishing in areas with low productivity and low tree cover. This is coherent with the ecology of rabbit, as the species avoids forested areas and prefers open spaces with shrubs and pasture that are typically dry during Mediterranean summer (Blanco 1998).

Those areas with natural or semi-natural habitat ("dehesas") did not show a high abundance of rabbits in motorway verges. The effect of verges is higher in those areas that are already under human pressure, where they provide a refuge for the species in the landscape. Thus, it is possible that the relationship of rabbit with verges is more of tolerance rather than preference (Planillo and Malo 2013). We observed the higher abundances in agricultural areas, where crops provide food for rabbits (Barrio et al. 2013), but ploughing destroys their warrens and prevent them to establish in the fieldcrops (Calvete et al. 2004). Similar patterns of species inhabiting verges near agricultural areas have been also observed in mice species (Bellamy et al. 2000, de Redon et al. 2015).

Infrastructure variables had heavier weight and explained better rabbit abundance in motorway verges than environmental variables, as they accounted for more than 50% of total variance in data. Our analysis highlights the crucial role of road characteristics as important factors for animal response to infrastructures (Gomes et al. 2009, Farmer and Brooks 2012, Santos et al. 2013). The

most explanatory variables were the width of the verge and traffic volume. Traffic volume had a negative impact on rabbit abundance and increasingly so as the daily number of vehicles grows. The more vehicles, the higher the disturbance and probability of roadkill when individuals cross the road. In small roads, the low density of vehicles can have a positive effect in small mammal density, up to a threshold in traffic volume above which it becomes negative (Clarke et al. 1998, Seiler 2005, Gunson et al. 2011). As our survey was focused on motorways, our data already start at medium traffic levels. The high reproductive rate of rabbits is an advantage in this situation. Population can compensate mortality at medium traffic levels and such traffic will have higher impact on predators (Benítez-López et al. 2010, Rytwinski and Fahrig 2012). This combination of effects may create a predation release effect near motorways (Fahrig and Rytwinski 2009) that could explain smoother effect of traffic and medium levels observed in the quadratic relationship. For high traffic volumes, mortality by vehicles will have a larger effect on rabbit populations and the disturbance of the traffic will be higher, which may also reduce reproduction as a side effect of high stress levels (Navarro-Castilla et al. 2014).

The other main infrastructure variable, the width of verges, is a characteristic that depends on the design of the road. Wider verges provide larger habitat to the species and also allow individuals to stay further away from vehicles and avoid roadkill (Bellamy et al. 2000, de Redon et al. 2015). This is an interesting feature, as it can be easily managed during or after the construction of the infrastructure. When looking closely to our data, we detect a clear threshold at 10 m verges. Although the 19% of our verges had less than 10 m, only the 6% of transects with high rabbit abundance –more than 1000 pellets–, were in those narrow verges, suggesting that dense populations of rabbits generally occur at wider verges. Also,

vegetation in verges may play an important role. In our study, almost all verges had more than 25% of herbaceous cover and yet this variable was selected in two of the best models. Probably, the existence of some vegetation provides food for rabbits.

One potentially key feature associated with linear infrastructures is the embankment. The presence of embankment was not selected in our models, but it could be of importance in other areas where available ground for rabbits is scarce, as the embankment provides a good substrate for warren building (Gea-Izquierdo et al. 2005), and may benefit rabbit abundance in road verges (Barrientos and Bolonio 2009).

It is importance to note that the low variance explained by environmental variables in our analysis is a reflection of the high impact that infrastructure has in rabbit abundance. The surveyed motorways capture a wide range of environmental conditions and although the environment determines the survival of the species, motorway features were mostly responsible for the observed abundances. The observed response in rabbit abundance responds to two main gradients. One gradient is the preferred habitat for the species under natural conditions, the one determined by the environment. The other gradient will respond to the infrastructure characteristics, favoring rabbit populations in wider verges and areas with less traffic volume. Thus, maximum rabbit abundance occurs in areas of fine environmental conditions that have the optimal infrastructure characteristics. Another interesting effect is that infrastructure somewhat extends the distribution of the species in human environments, allowing rabbit populations to establish in areas where habitat disturbance would prevent it, like along intensive agricultural landscapes.

The extrapolation of our results to other species should be made with caution. However, as rabbits are widespread and can be found in all continents (Lees and Bell 2008), we feel that our results will have widespread applications in many countries. In addition, the same recommendations could be extended to other species inhabiting road verges, after the examination of each case. Anyhow, monitoring studies should follow to assess the real outcome and to improve initial measures for any species.

Although it is impossible to prevent human disturbance in many landscapes, our results show that species response is modulated to a high degree by features that can be modified and therefore, used in management to avoid undesirable outcomes and allow a human growth more compatible with biodiversity protection (McDonald et al. 2008). Also, as resources are limited, we should prioritize those areas with higher potential for conflict or where measures can be more effective (Malo et al. 2004b, Farmer and Brooks 2012). This will be the case, for example, of road verges in agricultural areas (this study, Bellamy et al. 2000, de Redon et al. 2015). If desired, the verge management may be counteracted by allowing the species to establish in the landscape, for example, leaving uncultivated areas between fieldcrops (de Redon et al. 2015) or maintaining suitable areas for predators and preys far from roads (Gomes et al. 2009).

In summary, it seems that controlling the width of the verges may be effective to reduce or increase rabbit populations near motorways as long as the environmental conditions are not too extreme for the species. A distance of 10 m from the road surface to perimeter fence seems to be threshold for high density of rabbits. In areas where verges may act as corridors for invasive mammals (Bennett 1990, Ramp et al. 2006), it is possible to make them less attractive by minimizing

the right of way and thus, reducing the available area. On the other hand, in those areas where roadside could be beneficial for the species (Sabino-Marques and Mira 2011, Ascensão et al. 2012) we could favour species abundance by maintaining wide verges with some vegetation. Also favouring not palatable plants or completely removing vegetation from verges will discourage herbivorous species (Bellamy et al. 2000, Sabino-Marques and Mira 2011). Other common measures, such as the building of a perimeter fence, seem to be ineffective for small species, as we found in our comparison between transects inside and outside the fence.

Finally, a good alternative to verges could be the conservation of patches of natural vegetation away from the motorway, that will preserve the species and also act as corridors between established populations (de Redon et al. 2015), as species prefer areas with less disturbance than motorway verges when available (Planillo and Malo 2013).

3.5 Acknowledgements

A.P. was supported by a PhD grant of the Basque Government. This study forms part of the CENIT-OASIS Project funded by a consortium of companies supported by the Centro para el Desarrollo Tecnológico e Industrial of the Spanish Ministry of Science and Innovation (CENIT-2008 1016). The Comunidad de Madrid, together with the European Social Fund, supports the TEG research group through the REMEDINAL-3 Research Network (S2013/MAE-2719).

3.6 Supporting material

Table 3.4. Values of all variables measured in the study area, divided into environment related variables and infrastructure related variables.

	Max	Min	Mean	SD
Pellets	4662.25	0	684.59	954.41
ENVIRONMENT				
Herb cover (%)	100	2.46	54.48	30.56
Shrub cover (%)	24.28	0	2.69	4.7
Crop cover (%)	92.06	0	28.42	33.47
Tree cover (%)	60.09	0	5.13	10.72
Unprod cover (%)	60.66	0	9.29	9.59
NDVI	0.02	-0.07	-0.03	0.02
Dist. water (m)	5767.18	72.88	2503.71	1566.44
Altitude (m)	1312.5	810.5	1069.55	142.04
INFRASTRUCTURE				
Verge width (m)	79.5	1	18.37	11.21
Herb cover (%)	100	7.5	68.12	29.22
Shrub cover (%)	72.5	0	13.2	18.11
Tree cover (%)	90	0	10.06	20.09
Unprod cover (%)	80	0	8.63	15.64
adt (vehicles/day)	31283.98	6293.64	18726.83	10337.8

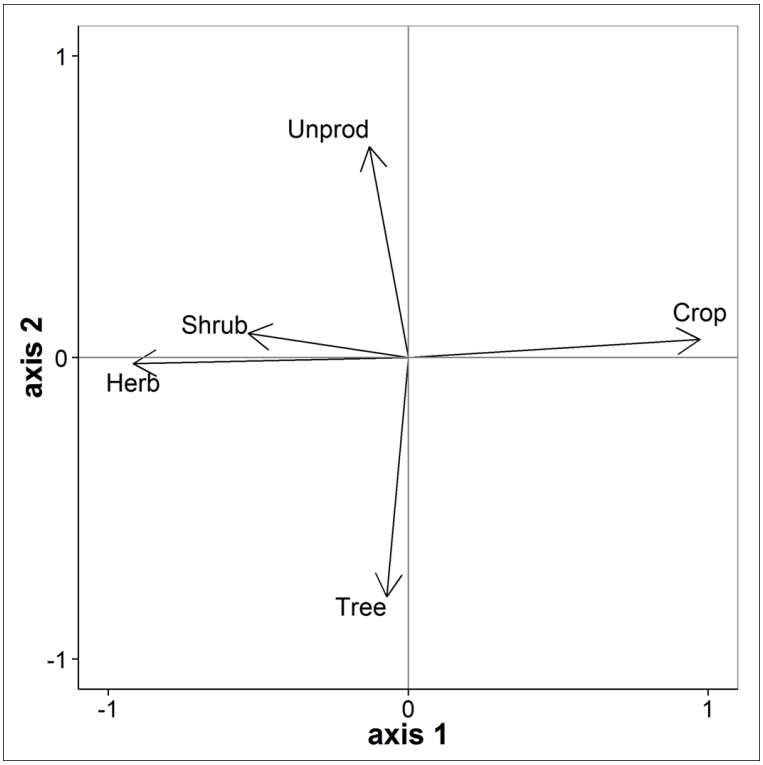

Figure 3.4. Factor loadings in the PCA for vegetation. Axis 1 explained 42% and created a gradient from plots with herbaceous (herb) and shrub vegetation to points dominated by crops. Axis 2 explained 23 % of variance and separated unproductive (unprod) points from points with tree vegetation.

Chapter 4

Transport infrastructure shapes foraging habitat in a Raptor community

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Abstract

Transport infrastructure elements are widespread and increasing in size and length in many countries, with the subsequent alteration of landscapes and wildlife communities. Nonetheless, their effects on habitat selection by raptors are still poorly understood. In this paper, we analyzed raptors' foraging habitat selection in response to conventional roads and high capacity motorways at the landscape scale, and compared their effects with those of other variables, such as habitat structure, food availability, and presence of potential interspecific competitors. We also analyzed whether the raptors' response towards infrastructure depends on the spatial scale of observation, comparing the attraction or avoidance behavior of the species at the landscape scale with the response of individuals observed in the proximity of the infrastructure. Based on ecological hypotheses for foraging habitat selection, we built generalized linear mixed models, selected the best models according to Akaike Information Criterion and assessed variable importance by Akaike weights. At the community level, the traffic volume was the most relevant variable in the landscape for foraging habitat selection. Abundance, richness, and diversity values reached their maximum at medium traffic volumes and decreased at highest traffic volumes. Individual species showed different degrees of tolerance toward traffic, from higher abundance in areas with high traffic values to avoidance of it. Medium-sized opportunistic raptors increased their abundance near the traffic infrastructures, large scavenger raptors avoided areas with higher traffic values, and other species showed no direct response to traffic but to the presence of prey. Finally, our cross-scale analysis revealed that the effect of transport infrastructures on the behavior of some species might be detectable only at a broad scale. Also, food availability may attract raptor species to risky areas such as motorways.

4.1 Introduction

The impact of human modified landscapes on wildlife is an important but yet not well understood issue. Human disturbances change community composition and modify ecosystems (Devictor and Robert 2009, Rodewald et al. 2011, Le Viol et al. 2012). Among human alterations, elements of the transport infrastructure (i.e. roads) are a common feature in many countries, and they are likely to increase in number, extent and intensity of use all over the world in the near future. Transport infrastructure alters the surrounding environment with varying effects on vertebrate species (Forman and Alexander 1998, Fahrig and Rytwinski 2009). Species may be affected positively by the creation of new habitat or corridors (Bennett 1990, Rosa and Bissonette 2007, Ruiz-Capillas et al. 2013b), or negatively by direct mortality, fragmentation, noise disturbance, or habitat loss (Trombulak and Frissell 2000, Forman et al. 2003, Jaeger et al. 2005). Also the extent of these effects is not restricted to areas beside roads and can spread up to several kilometers for large species (Benítez-López et al. 2010, Basille et al. 2013).

Many studies have focused on the effect of roads on terrestrial vertebrates, but few of them were centered in flying species (Fahrig and Rytwinski 2009, Benítez-López et al. 2010). Nonetheless, transport infrastructure can pose a serious threat to many birds. Roads might seem attractive to some species as they can provide valuable resources for birds like perches, food, or nesting sites in shrubs in their verges or adjacent structures (Erritzoe et al. 2003, Morelli 2011 and references below). However, birds close to roads suffer negative population effects such as decreased breeding success or direct mortality by vehicle collision (Reijnen et al. 1996, Mumme et al. 2000, Gomes et al. 2009). In general, the more mobile the bird species, the higher the road impacts (Rytwinski and Fahrig 2012).

In this context, the response of diurnal raptors to roads is of special interest. Most raptor species are listed under several categories on the IUCN red list (IUCN 2013), they are targets of conservation programs, and they also have an important ecological role in ecosystems as top predators. Moreover, habitat alterations are among the main factors behind the decline of raptor populations over the last years (del Hoyo et al. 1994, Madroño et al. 2004, Carrete et al. 2009). Transport infrastructure effects have been studied at local scales, with some species selecting roads due to food or perching site availability (Meunier et al. 2000, Dean and Milton 2003, Bautista et al. 2004). Although traffic volume might change the response of raptors to roads, decreasing road use of some of them when traffic increases (e.g. large species, Bautista et al. 2004). However, few studies have analyzed the habitat selection of raptors at broader spatial scales, and raptor community response to transport infrastructure at the landscape scale remains unclear. Contrary to local scale studies, Knight and Kawashima (1993) failed to find differences between roads and control sites for red-tailed hawks (*Buteo jamaicensis*), and scavenger raptors showed different responses depending on the species (Lambertucci et al. 2009).

Species can select habitat at different scales, from landscape to local scale. Although sometimes different scales can share important variables (Barrientos and de Dios Miranda 2012), it is expected that the main response of the species to habitat features occurs at the landscape scale, according to the hierarchical habitat selection (HHS) hypothesis (Rettie and Messier 2000, Basille et al. 2013). Thus, to understand raptor habitat selection, we first need to understand the important factors at the landscape scale. Accordingly, most studies have focused on the effects of factors at this scale related to habitat, food availability, competition, or even human activities (Cody 1985, Sánchez-Zapata and Calvo 1999, Palomi-

no and Carrascal 2007). However, two interesting questions remain unexplored: how important are road effects in comparison to other factors for raptors' habitat selection, and whether the raptors' response to roads detected at the local scale is comparable to that at the broad scale..

In order to disentangle the effects of the transport infrastructure on raptor community, we studied the foraging habitat selection of diurnal raptors during their daily movements. We analyzed raptors' responses to roads with different levels of traffic, including also other variables linked to habitat selection such as habitat structure, food availability and interactions with other potentially competing species. Our objectives were to identify the most relevant variables for the foraging habitat selection of raptors at landscape scale and to compare the response of raptors to traffic detected at a landscape scale with that observed at a local scale. Based on the existing literature for habitat selection and road effects on raptors, our hypotheses were: 1. Transport infrastructures will be among factors affecting foraging habitat selection of raptors at landscape scale, as it is been shown for some species at local scales (Meunier et al. 2000, Dean and Milton 2003, Bautista et al. 2004). We predict that the presence of roads will affect raptor behavior, with larger effects for higher traffic levels, due to the disturbing effect of traffic (Forman and Alexander 1998). 2. The change in the raptor community is expected to occur both in species composition and abundance (McKinney 2006, Devictor et al. 2007). We predict that the most tolerant and opportunistic species will be more abundant near infrastructures, whereas the less tolerant will avoid them despite the availability of resources. 3. Those species strongly affected by transport infrastructures will show similar responses to traffic across scales (Rettie and Messier 2000). We predict that only individuals from opportunistic tolerant species will select transport infrastructures at both scales, while less tolerant species will

avoid them at least at one of the scales to minimize the perturbation of the infrastructure (Basille et al. 2013).

4.2 Methods

4.2.1 Study Area

The study was carried out in an area of 3600 km2 in southern Castilla y León Region, Central Spain (Fig. 4.1A). It is a rural area, with an average population density of 25.5 inhabitants per km2. The climate is continental Mediterranean, with cold winters and dry summers, and average annual precipitation about 490 mm. The landscape can be categorized into three main formations: agricultural lands of non-irrigated cereals, pastures with oak trees used for cattle grazing ("dehesas"), and patches of natural vegetation, characterized by holm oak forests (Quercus ilex) and Mediterranean shrub formations. Areas of high conservation value included in the European Natura 2000 Network extend over 500 km^2 in the study area.

Four motorways cross the area, characterized by 4 to 6 traffic lanes and a speed limit of 120 kmh^{-1}. Motorways can be divided into medium-traffic motorways (AP-51, AP-61 and A-50), with an average daily traffic (ADT) of 6,500-8,000 vehicles and an approximate length inside the study area of 80 km, and high-traffic motorways (AP-6), with an ADT of 20,000 vehicles and an approximate length of 65 km. In addition, there are several 2-lane roads connecting small cities and villages, with speed limits of 90-100 kmh-1. Roads usually support less traffic volume and their ADT ranged from 1,000 to 3,300 vehicles (with some punctual busy stretches up to 6,000 vehicles). AP-51, AP-61 and AP-6

motorways belong to ABERTIS-IBERPISTAS that granted permission to work on them.

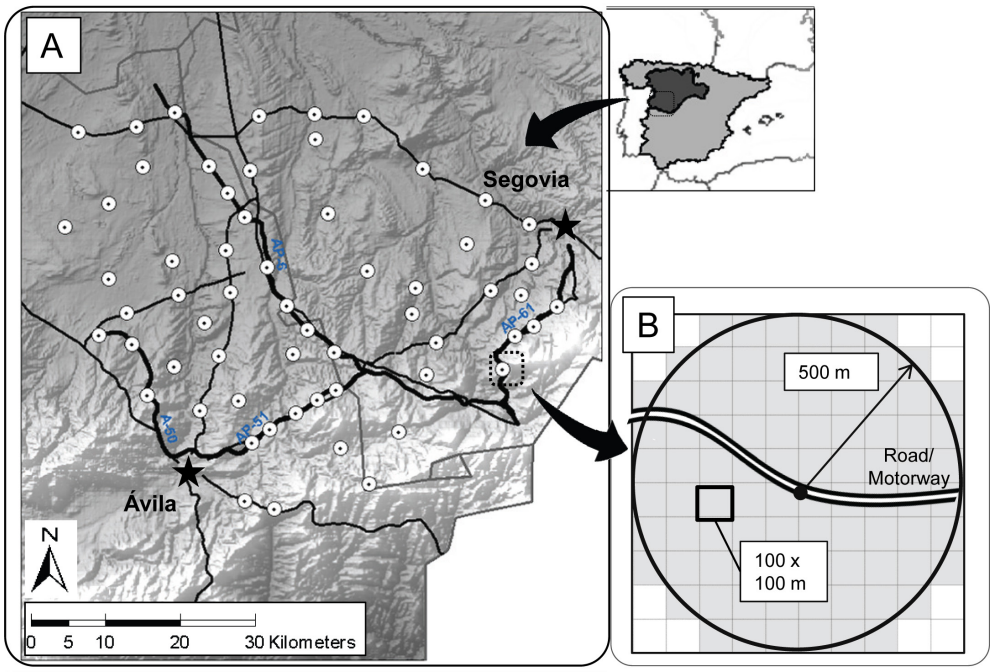

Figure 4.1. Study area and survey design. A. Location of the study area and the observation points (white dots) in Castilla y León Region, Central Spain. Thin black lines represent 2 lane roads and bold black lines represent motorways. Main cities in the area are displayed with a star. Base map downloaded from Instituto Geográfico Nacional de España (IGN, www.ign.es). B. Detail of a sampling point either in a road or motorway, and the grid used for local scale analyses (shaded area) of 100 x 100 m cells inside the 500 m radius.

4.2.2 Survey design and data collection

Data on raptor foraging habitat selection was taken on 60 sampling plots distributed throughout the study area, 20 along motorways, 20 along main two-lane roads and 20 in control sites. We consider control sites as those that were at least 3

km away from roads and motorways. Sampling plots were randomly distributed, in places with good visibility, always next to the asphalt surface on roads and motorways, and at least 3 km from the nearest sampling plot to ensure independence of the observations (Fig. 4.1A). Each sampling plot was visited 4 times, two times in the breeding season (June 2010 and 2011), and two times in the winter season (January-February 2011 and 2012).

The sampling plot was defined as the area in a radius of 500 m from the observer's position, with road and motorway plots always centered on the infrastructure. During each visit, one observer recorded all the individuals showing foraging behavior inside the sampling plot during 30 minutes. We assumed raptors to be foraging when they were actively hunting or searching the ground by soaring at low altitude. Surveys were conducted during the whole day, three plots during morning and three during afternoon, one from each zone and alternating the order of the zones from one day to the next to avoid hour bias. To avoid sampling biases due to weather dependent factors (Bibby et al. 2000), or to changes in human disturbances on weekends (Bautista et al. 2004), the field work was restricted to windless and rainless work days starting 2-3 hours after sunrise and finishing two hours before sunset.

A. Variables used for landscape scale analysis

At a landscape scale, we analyzed the foraging habitat selection of raptors both at community and species-specific levels, with special focus on the traffic effects.

Response variables. Individuals were identified at species level whenever possible. For the community level responses, the number of individuals detected

per plot in each survey was taken as the relative abundance and the number of species as the relative richness. We also estimated the Shannon-Wiener diversity index for each plot. For the species level response, we analyzed the abundance of the species with more than 40 observations: red kite (*Milvus milvus*), black kite (*Milvus migrans*), booted eagle (*Hieraaetus pennatus*), common buzzard (*Buteo buteo*), griffon vulture (*Gyps fulvus*), cinereous vulture (*Aegypius monachus*) and kestrels (Supplementary Table 4.4). During the breeding season two kestrel species (*Falco tinnunculus* and *Falco naumanni*) share the area and their determination at the species level was not always certain. Therefore both species were pooled together.

Predictor variables. For each sampling plot, we estimated seven predictor variables based on literature for foraging habitat selection, and that were related to the infrastructure (see the list and a brief description in Table 4.1).

The sampling plots belonged to one of the three categories of traffic infrastructure types used for the stratification of the survey design. The stratification carried out for sampling in control, road and motorway sites reflects a parallel gradient in two underlying variables: traffic volume and speed limit. Thus, the highest traffic volumes occur in motorways, which also have the highest speed limit (120 kmh^{-1}). The opposite situation appears in control sites (no traffic and '0' speed limit), while roads have intermediate traffic levels with speed limits around 90 kmh^{-1} (as we only used main roads for our study). Since traffic volume has been shown to have an effect on the response of some raptor species to roads (Bautista et al. 2004), we decided to use traffic volume, measured as average daily traffic (ADT), to test the general effect of transport infrastructure. We assigned each sampling plot to a habitat type (see Table 4.1 for details), since some species

Table 4.1. Predictor variables for the landscape scale analysis. For each predictor there is a code used in the statistical analysis and a brief description. Values are presented as mean ± SE and range for quantitative variables, and levels for categorical factors.

Code	Predictor Description	N	Values (mean ± SE)
ADT	Average daily traffic. Obtained each natural year as an estimate of the number of vehicles a day that drive through each sampling plot. Continuous variable.	180	4888 ± 371.47 (0 – 20850)
Habitat	Main vegetation of each sampling point. Factor.	60	Crops, Monte1, Pines, Unproductive, Mixed2.
D.vill	Distance to the nearest village. Continuous variable, Log-transformed.	60	3.11 ± 0.04 (2.19 – 3.83)
Rabbits	Relative rabbit abundance. Sum of rabbit pellets in 10 plots of 0.5 m^2, evenly spaced along a 1km transect inside the sampling plot. Continuous variable, Log-transformed. Obtained during breeding season.	120	1.03 ± 0.06 (0 – 2.58)
Micros	Relative small mammal abundance. Sum of warrens found in 10 plots of 5 m radius, evenly spaced along a 1km transect inside the sampling plot. Counts. Obtained during breeding season.	120	14.13 ± 1.14 (0 – 126)
HT.Rkill[a]	Distance to the nearest roadkill hotspot in high-traffic motorway. Continuous variable, Log-transformed.	120	3.88 ± 0.11 (0.92 – 4.44)
MT.Rkill[a]	Distance to the nearest roadkill hotspot in medium-traffic motorways. Continuous variable,Log-transformed.	120	4.15 ± 0.05 (2.66 – 4.67)
season	Controlling variable. Season of each survey. Factor.	240	Breeding, Winter
time	Controlling variable. Time of the day of the survey. Factor.	240	Morning, Afternoon
visib	Controlling variable. Proportion of the sampling plot fully visible due to terrain constrains. Proportion.	60	0.92 ± 0.01 (.56 – 1)

[a]Distances to roadkill hotspots were calculated in winter and breeding season independently, based on roadkill database and two year roadkill monitoring (unpublished data).

[1] Holm oak forest and dehesas with high shrub cover.. [2] Sampling points with no clear dominance of any vegetation classes.

have shown preference for certain habitats, for example, kestrels usually select open areas (del Hoyo et al. 1994), common buzzards prefer forested areas (Sánchez-Zapata and Calvo 1999), and black kites avoid woodland (del Hoyo et al. 1994, Sergio et al. 2003). The distribution of habitat types did not show significant differences among the survey traffic strata (χ^2 = 8.55, p = 0.359 computed with a 2000 replicate Monte Carlo simulation), so we discarded a possible confounding effect of the habitat (e.g. one habitat type being more abundant in control sites than in motorways). We also calculated the distance to the nearest village because the proximity of urban areas can alter the habitat use of the species, for example black kites, booted eagles and kestrels have shown preference for villages due to availability of anthropogenic resources like garbage or anthropophilic prey, discards from farms and nesting sites in old buildings (Veiga 1986, Marío Díaz et al. 1996, Sergio et al. 2003, Palomino and Carrascal 2007), while common buzzards show avoidance of villages, probably due to excessive disturbance (Palomino and Carrascal 2007).

Road verges can act as refuges for small mammals and increase their abundance (Bennett 1990, Ruiz-Capillas et al. 2013b). Once a year, we estimated relative abundance indices of two main preys: small mammals (mainly voles) and rabbits. This survey was carried out during breeding season, as it is the productivity peak for those species. Both taxa are common prey for red and black kites, buzzards, and booted eagles (Delibes and García 1984, Veiga 1986, Beja et al. 2009). Voles are also the main prey for common kestrels (Marío Díaz et al. 1996), and rabbit carcasses can be important in the diet of black vultures, and also consumed by griffon vultures (del Hoyo et al. 1994). Vultures are obligate scavengers, although other species in the area also use carrion, such as kites and buzzards (del Hoyo et al. 1994).To test the effect of the availability of carrion, we identified

roadkill hotspots in motorways following the method in Malo et al. (2004b). This method detects clusters of animal collisions along a road and defines hotspots as those stretches containing a higher number of roadkills than would be expected from a random distribution (poisson distribution with the observed mean). Data on location of roadkills were obtained from a database provided by the motorway management agency and from a monthly survey carried out during two years. The total roadkill data showed very few ungulate casualties, with rabbits being the most common roadkilled species (unpublished data). Since the traffic density might affect the behavior of some raptors (Bautista et al. 2004), we divided roadkill hotspots into two categories: those affected by medium traffic and those affected by high traffic volume. Distances to both types of hotspots were included in the analyses as separate variables to account for different responses to carrion depending on the traffic density.

We also measured three variables that could influence the flying behavior of raptors and their detectability, and included them in the statistical models when necessary: time of day, visibility and season (controlling variables in Table 4.1). Season was only included for those species that were present in the study area all year round (Supplementary Table 4.4).

All distance measurements and habitat assessment were done in ArcGIS 9.3, using public cartography and aerial photography from Castilla y León Region (Junta de Castilla y León 2011).

B. Estimation of infrastructure use index

Within road and motorway sampling plots, we analyzed the response of raptors

to the infrastructure itself against surrounding habitat (hereafter denoted as "infrastructure use") to compare it with the response of the species to the infrastructure at the landscape scale. We superimposed a 100 x 100 grid to the plot (500 m radius) (Fig. 4.1B), and we defined an infrastructure use index based on the proportion of sightings for each individual located on asphalt cells related to the total amount of sightings for that individual. Total sightings per individual ranged from 1 to 6, depending on the time the raptor spent inside the sampling plot.

Cells of the grid traversed by the transport infrastructure were classified as "asphalt cells" and the rest were classified as "non-asphalt cells". During each survey, the observer followed each raptor and recorded its position in the grid every five minutes. As the raptors were flying at low altitude (foraging flight, see above), their position was determined on aerial photographs and supported on easily recognizable landmarks, such as lonely trees or field borders that ensure enough accuracy in the 100 x 100 grid.

4.2.3 Statistical analyses

A. Landscape scale

We first analyzed trends in raptor community as a whole by multidimensional scaling analysis (MDS). This technique creates new synthetic variables (dimensions) from the data on species presence and abundance, and projects the sampling plots on those new variables (Quinn and Keough 2002). Sampling plots with similar community composition will appear nearby, while plots with different species or abundances will be apart. The distance matrix used to build the MDS was calculated using the Bray-Curtis index (Quinn and Keough 2002).

This index is commonly used in community analyses, as it uses information on abundance of each species to compute the distance matrix. We built a MDS with two dimensions to allow easier interpretation of results, after checking in the scree-plot of stress values that the inclusion of a third one did not improve stress noticeably (stress value for two dimensions: 0.25). Influence of each species was calculated by Spearman rank correlation of species abundance with both dimensions. Seasonal changes in raptor community and the effect of traffic, represented in a synthetic way by the types of infrastructure (control, road, motorway), were tested by a multivariate analysis of variance (MANOVA) and Tukey HSD post hoc tests. For these analyses, the locations of sampling plots in the two dimensions were used as response variables.

The relative relevance of transport infrastructure on foraging habitat selection of raptors was analyzed for the three community variables (diversity, abundance and richness), and for individual taxa by generalized linear mixed models (GLMMs, Bolker et al. 2009, Zuur et al. 2009), which were ranked and selected based on information-theoretic criteria (Johnson and Omland 2004, Richards et al. 2011). For each response variable, we created a set of candidate models based on ecological hypotheses. The hypotheses were divided into three groups: (i) General habitat structure, including main vegetation types, distance to nearest village and traffic volume of sampling plots; (ii) Food availability, including natural prey abundance as well as road casualty carcasses; (iii) Interaction with other raptor species, only for the species-level analyses, and including among the predictors the abundance of the most common species in the area: *M. milvus, M. migrans* and *H. pennatus*. The traffic predictor (ADT) was included in quadratic form, as vertebrates may show a non-linear response to traffic (Clarke et al. 1998, Gunson et al. 2011). The candidate set of models for each response variable inclu-

ded models representing one or more hypotheses through different combinations of predictors, using only those predictors relevant to that response (as described in the "variables used for landscape analysis" section).

Prior to model fit, we tested for correlation between predictor variables using Spearman's rank correlation. No correlation higher than 0.7 was found, thus all variables were considered. We first explored the shape of the relationship between responses and predictors by generalized additive models (GAMs), using the full model and fitted smoothing splines with 3 degrees of freedom. Then, linearity was assessed by visual inspection of the partial residual plots (Crawley 2007) and when appropriate, logarithmic or quadratic transformations of predictor variables were applied. In order to avoid overparameterization, we included our controlling variables (season, time of day, visibility) only on those models for which they were informative.

GLMMs were fitted using gaussian error structure with identity link for the diversity models, and poisson error structure with log link for all the others. The identity of the sampling plot was used as random factor. When the response included a high number of zeros (*B. buteo, G. fulvus, A. monachus*), zero-inflated poisson (ZIP) distribution was used instead. ZIP distribution allows analyzing data that present more zeros than expected in a poisson distribution, avoiding potential overdispersion and bias in the parameter estimation due to the excessive number of zeros (Zuur et al. 2009). To model the ZIP distribution we followed the procedure used by Bolker et al. (Bolker et al. 2012), including a single constant term across the entire model to account for zero-inflation across the data set (Fournier et al. 2012, Skaug et al. 2012). For poisson GLMMs, overdispersion was tested and found close to 1 in all cases, thus no correction was applied.

Candidate models were compared and ranked using Akaike Information Criterion corrected for small sample size (AICc). A null model containing only the intercept was also included in the candidate set for comparison. For further analyses and interpretation, we selected models within < 2 ΔAICc, i.e. the difference to the AICc of the best ranked model, as all of them should be considered competitive for interpretation (Burnham and Anderson 2002). Within the selected models, whenever two nested models differing only in one parameter were selected, they were considered redundant and we only included the one with the lowest AICc value to avoid overrepresentation of predictors (Burnham and Anderson 2002, Arnold 2010, Richards et al. 2011).

Furthermore, we analyzed the relative importance of the explanatory variables for each response. The relative importance of a predictor was obtained by computing the summatory of Akaike weights (w_i) of the models where the predictor was present, using only the set of selected models. Therefore, a predictor that appears in all the selected models will have the maximum value of 1, meaning that it is the most important predictor for the respective response variable within the group of analyzed predictors. To interpret the effect of the explanatory variables, for each response we calculated the average model and obtained the average coefficients with shrinkage (Burnham and Anderson 2002). As this method does not provide a reliable estimate for the standard deviation of coefficients (Burnham and Anderson 2002), we decided to interpret coefficients with absolute values smaller than 0.01 as indicative of a lack of a relevant effect in biological terms.

B. Infrastructure use analysis

Within the sampling plots, we evaluated the preference or avoidance of the in-

frastructure for each observed individual of a single species using a use versus availability approach (Manly et al. 2002, Johnson et al. 2006, Northrup et al. 2013). The index of infrastructure use was built based on proportions, so we used GLMMs with binomial error distribution and logit link, including sampling plot identity as random factor. As we did in the previous analyses, we first checked for effects of controlling variables on the responses and included them when necessary. As our goal was to compare the response of the individuals to the infrastructure when they are in its proximity with the response of the species at the landscape scale, we only included traffic as predictor. We used the number of sightings per individual as a weighting variable to account for the variability in this measure (sightings ranging from one to six, see above). In order to test for lower or higher use of asphalt cells than their availability, the model intercept was forced to be the actual proportion of asphalt cells in each plot, representing the expected value if raptors used them randomly.

All statistical analyses were performed using R statistical software 3.0.3 (R Core Team 2014). GAM, GLMM and ZIGLMM models were fitted using mgcv (Wood 2011), lme4 (Bates et al. 2014), and glmmADMB packages (Skaug et al. 2012), respectively. AICc values and averaged coefficients were obtained with the MuMIn package (Barton 2014). All values presented in the results section refer to mean ± standard deviation, unless specified otherwise.

4.3 Results

We recorded 743 raptors belonging to 18 different species (Supplementary Table 4.4). Nine species were observed in less than 15 occasions, and therefore they

were included in the community-level analysis but discarded for the species-level analysis. One individual of rough-legged buzzard *Buteo rufinus* was also detected but removed from the dataset because it is considered a rare species in the area.

There was significant difference in abundance between types of plots (χ^2 = 9.24, df = 2, p = 0.009), but not for richness or diversity (χ^2 = 4.99, df= 2, p = 0.117; F = 1.59, df = 2, p = 0.213, respectively). We found a total of 187 raptors in control plots, 291 raptors in plots located near roads, and 265 raptors in motorways, with a similar significant abundance pattern in analyzed species (Fig. 4.2). The mean richness per plot was 1.63 ± 1.38 in control sites, 1.95 ± 1.35 in roads, and 2.05 ± 1.34 in motorways. In a parallel way, the diversity index was 0.46 ± 0.06 in control sites, 0.54 ± 0.06 in roads, and 0.60 ± 0.06 in motorways.

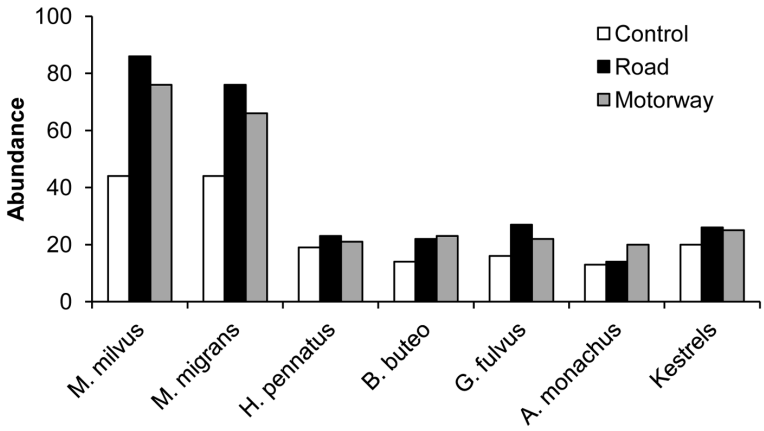

Figure 4.2. Species abundance. Total abundance of common raptor species in sampling plots of each type of transport infrastructure. Details for all the species detected in the study can be seen in Supplementary Table S4.1.

4.3.1 Landscape scale analyses

The MDS analysis showed differences in raptor community composition linked to the presence of transport infrastructure and season (Fig. 4.3). There was a clear distinction in the community structure between breeding and winter seasons ($F = 157.27$, $p < 0.001$), and a significant interaction between season and transport infrastructure ($F = 2.83$, $p = 0.025$). In each season, we found the largest differences in community composition between road plots and control sites, being significant only in winter ($p = 0.041$), but not in all other cases ($p > 0.05$), and with motorway plots in an intermediate position (Fig. 4.3).

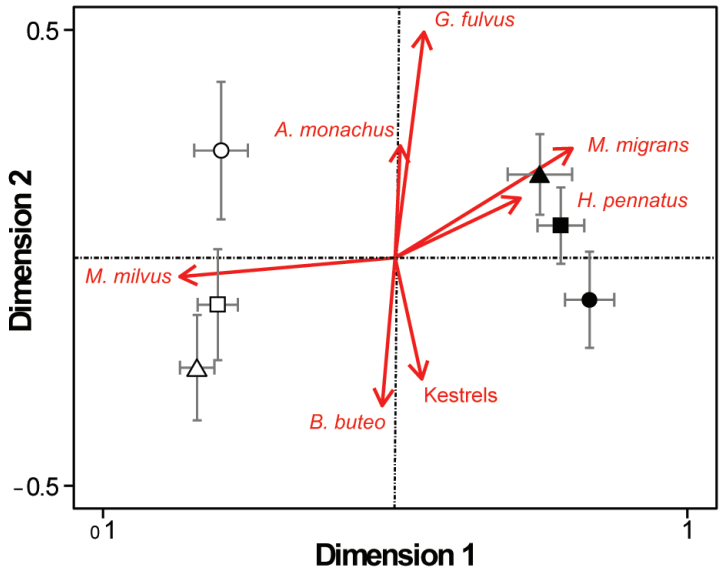

Figure 4.3. Two dimensional ordination (MDS) of general trends in raptor community. Species composition of sampling plots represented by transport infrastructure types and season (mean ± SE of position in dimensions 1 and 2). The influence of most abundant species is also represented with arrows of length and direction obtained from their correlation with the axes. Circles represent control plots, triangles road plots, and squares motorway plots. Solid symbols for breeding season and empty symbols for winter. Stress value of the ordination of 0.25.

Regarding the habitat hypothesis at the community level, the most informative predictor variable for diversity, abundance and richness of raptors was the traffic volume (ADT) (Table 4.2. For the full list of models, see Supplementary Table 4.5). All three responses showed a quadratic response to traffic, reaching the maximum with medium traffic levels and then decreasing at highest traffic volume, especially in the case of diversity (Fig. 4.4, coefficients in Supplementary Table 4.13).

In the species-specific analyses more than one model was selected for all taxa except booted eagles (Table 4.2). The variable that was selected for most species was the traffic volume –selected in 5 out of 7 species–, followed by the abundance of natural prey –selected in 4 species–, and the distance to anthropogenic areas, both villages and roadkill hotspots –selected in 3 species–. Also the null model was selected for common buzzard and griffon vulture, suggesting that the predictor variables had low explanatory power for them (for the full list of models for each species see Tables 4.6 to 4.12 in supplementary material).

The Akaike weights of each explanatory variable for each response can be seen in Fig. 4.4 (see Supplementary Table 4.14 for the whole set of averaged coefficient values). Response to traffic varied among species, both in relative importance and coefficient signs. Both kites and kestrels showed increasing abundance with low to medium traffic volumes ($\beta_{ADT,M.milvus}$ = 1.301, $\beta_{ADT,M.migrans}$ = 0.911, $\beta_{ADT,kestrels}$ = 0.777). However, red kite and kestrels abundance decreased at higher traffic values ($\beta_{ADT2,M.milvus}$ = -0.785, $\beta_{ADT2,,kestrels}$ = -0.619), and only black kite remained abundant ($\beta_{ADT2,M.migrans}$ = 0.279). In the case of common buzzards and griffon vultures, although they had slightly higher abundance in areas with medium-low traffic than control sites ($\beta_{ADT,B.buteo}$ = 0.101, $\beta_{ADT,G.fulvus}$ = 0.215),

Table 4.2. Selected models for foraging habitat selection with traffic effects at the landscape scale. Models are presented based on the ecological hypothesis they support: (0) intercept only, (i) Habitat structure, (ii) Food availability, (iii) Interaction with other species. A full list of models can be found in supplementary material (S2-S11 Tables).

Response	Hypothesis supported by model	AICc[1]	ΔAICc	wi[2]
Community-level				
Diversity	(i) Traffic volume	324.62	0.00	0.501
Abundance	(i) Traffic volume	1043.26	0.00	0.524
Richness	(i) Traffic volume	778.79	0.00	0.475
Species-level				
Red kite (Milvus milvus)	(i) Traffic volume	574.63	0.00	0.244
	(i)Distance to village	574.66	0.03	0.241
Black kite (Milvus migrans)	(ii) Anthropogenic food from roadkills	387.76	0.00	0.246
	(i) and (ii) Traffic volume and anthropogenic food from roadkills	389.00	1.24	0.132
Booted eagle (Hieraaetus pennatus)	(ii) Rabbit and micros abundance	238.17	0.00	0.430
Common buzzard (Buteo buteo)	(iii) Interaction with M. migrans	281.44	0.00	0.147
	(ii) and (iii) Rabbits and micros abundance and interaction with M. migrans	281.59	0.14	0.137
	(0) Intercept only model	282.21	0.77	0.100
	(iii) Interaction with M. milvus	282.80	1.36	0.075
	(i) Distance to village	283.38	1.94	0.056
	(i) and (iii) Traffic volume and interaction with M. migrans	283.39	1.95	0.056

Response	Hypothesis supported by model	AICc[1]	ΔAICc	wi[2]
Kestrels (Falco tinnunculus, F. naumanii)	(i) Distance to village	234.24	0.00	0.306
	(ii) Micros abundance	324.38	0.14	0.286
	(i) Traffic volume	324.81	0.57	0.231
Griffon vulture (Gyps fulvus)	(0) Intercept only model	303.97	0.00	0.267
	(ii) Anthropogenic food from roadkills	304.70	0.74	0.185
	(i) Traffic volume	305.47	1.50	0.126
	(ii) Rabbit abundance (carcasses)	305.61	1.64	0.117
Cinereous vulture (Aegypius monachus)	(ii) Anthropogenic food from roadkills	239.90	0.00	0.376
	(ii) Anthropogenic food from roadkills and Rabbit abundance (carcasses)	240.08	0.18	0.344

1AICc = Akaike Information Criterion value corrected for small sample size.

2wi = Akaike weights of the models in the full set of candidate models.

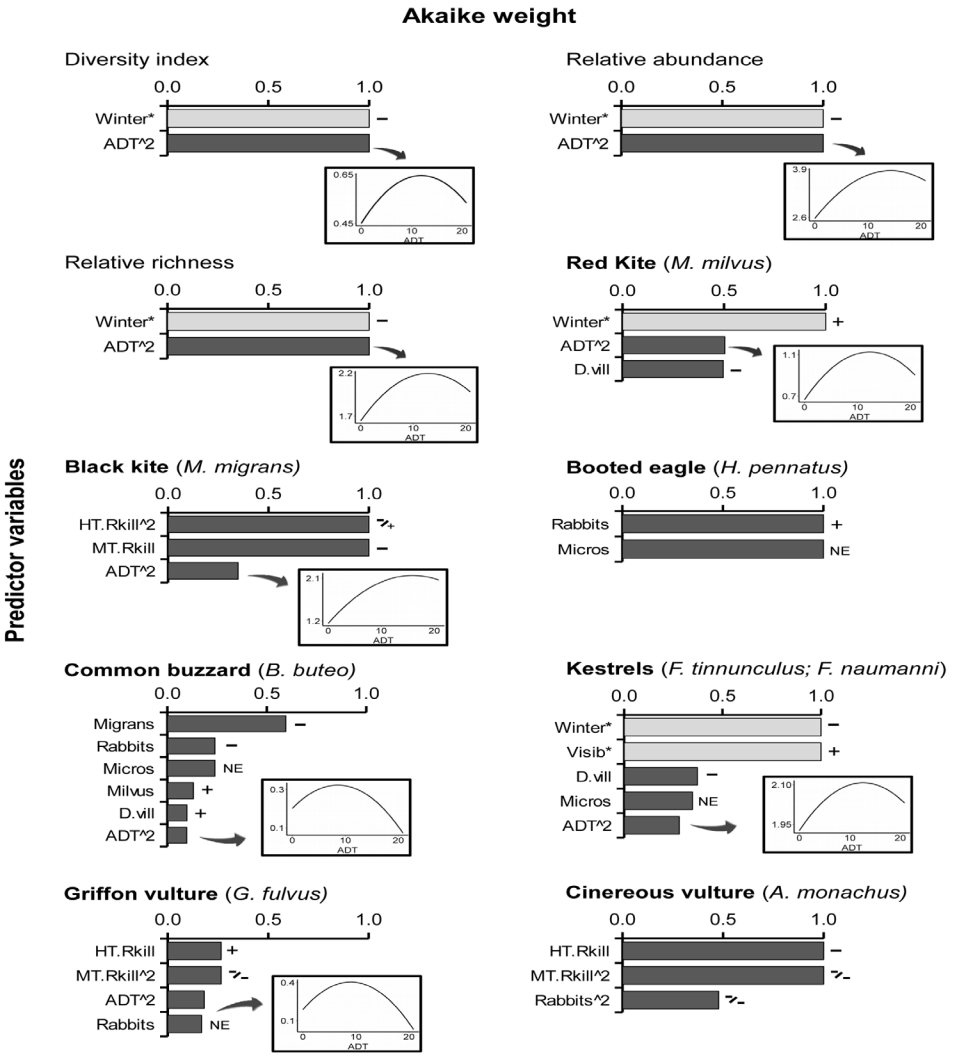

Figure 4.4. Relative importance of foraging habitat selection predictor variables at landscape scale. Akaike weights of predictors for each response variable in the community level analyses (diversity, abundance, richness) and the species-level analyses. Controlling variables are identified by asterisks and colored in light grey. The sign of the effect in the final average model is shown as positive (+) or negative (-). Variables in quadratic form are identified with "^2" and the symbols correspond to the simple and quadratic form, respectively. Variables with an averaged coefficient close to zero (≤|0.01|) are marked as "no effect" (NE). When the variable ADT is selected, the curve showing its effect in the response is also included. In this case, the x axis is always the amount of traffic (ADT), from 0 to 20.850 vehicles a day, and the y axis represents the value of the response variable. For a definition of the variables see Table 4.1 and the values of the averaged coefficients are in Supplementary Tables S4.10 and S4.11.

their abundance decreased quickly with increasing traffic amounts ($\beta_{ADT2,B.buteo}$ = -0.199, $\beta_{ADT2,G.fulvus}$ = -0.762).

The roadkill hotspots were selected as important variables for species with the most marked scavenger behavior. Cinereous vultures were more abundant near hotspots in both medium and high traffic areas ($\beta_{L.HTRkill}$ = -0.157, $\beta_{L.MTRkill}$ = -9.300, $\beta_{L.MTRkill2}$ = -6.971). Black kites and griffon vultures were abundant near roadkill hotspots located in medium traffic areas ($\beta_{L.MTRkill,M.migrans}$ = -0.082, $\beta_{L.MTR-kill,G.fulvus}$ = -0.299, $\beta_{L.MTRkill2,G.fulvus}$ = -2.098), but avoided those in high traffic areas areas ($\beta_{L.HTRkill,M.migrans}$ = -0.034, $\beta_{L.HTRkill2\ ,M.migrans}$ = 3.379, $\beta_{L.HTRkill,G.fulvus}$ = 0.012).

Red kites and kestrels selected areas close to villages, and common buzzards avoided them. Finally, in the case of booted eagle, no variable related to traffic was selected and its abundance was directly related to the abundance of rabbits (Fig. 4.4).

4.3.2 Infrastructure use analysis

We analyzed the local behavior of individuals when observed inside infrastructure plots for 527 individuals belonging to the same taxa analyzed at the landscape scale. When the behavior was analyzed within 500 m of the infrastructure (radius of the sampling plot), red kites flew over the infrastructure more frequently than random for increasing traffic and griffon vultures avoided the infrastructure, especially with high traffic levels. For all other cases, we found no selection or avoidance of the infrastructure related to the amount of traffic (Table 4.3, for coefficients of the models see Supplementary Table 4.15).

Table 4.3. Raptors selection of asphalt cells within the sampling plots. Models for red kites, common buzzard and kestrel included the controlling variable season, and the model for black kites included the controlling variable time (all coefficients in S4.12 Table. (+ Preference, significant positive selection; - Avoidance, significant negative selection; NS = Indifferent, no significant effect found).

Species	ADT	ADT2
Red kite (*Milvus milvus*)	+	NS
Black kite (*Milvus migrans*)	NS	NS
Booted eagle (*Hieraaetus pennatus*)	NS	NS
Common buzzard (*Buteo buteo*)	NS	NS
Griffon vulture (*Gyps fulvus*)	-	-
Cinereous vulture (*Aegypius monachus*)	NS	NS
Kestrels (*Falco tinnunculus, F. naumanii*)	NS	NS

4.4 Discussion

We present the first comprehensive analysis of a diurnal raptor community aimed to compare the effects of transport infrastructure with other landscape factors in foraging habitat selection. The traffic volume of roads played a central role in the community metrics – diversity, abundance and richness –, and it was also the predictor selected most times in the species specific analyses, appearing in five out of seven species. As expected, individual species showed different degrees of tolerance toward traffic, from preference to avoidance, with the latter being especially strong for areas with high traffic volumes. In contrast, when comparing the response to traffic at different scales, we found no response to traffic for many species at the local scale. In our study, the effects of traffic were detectable at a broad scale, as predicted by the HHS hypothesis when analyzing the main habitat selection variables (Rettie and Messier 2000).

4.4.1 Effects of transport infrastructure on foraging habitat selection of raptors at landscape scale

For the community metrics no other explanatory variable than traffic was included in the models, thus indicating that traffic volume was the main feature driving raptor community foraging habitat selection. Community metrics summarize responses from all the species and they reflect the most common one. However, they might fail to reflect ecological difference among species in disturbed landscapes (Devictor and Robert 2009), and finding common trends could thus be a difficult task for community metrics. Nonetheless, by calculating species-specific models we were able to disentangle effects on individual species and to find some common trends.

Five out of seven species (71.4%) showed a direct reaction to traffic, although the explanatory power of this predictor should be considered low for the two species that also included the null (intercept only) model (Table 4.2). Many species increased their abundance near areas with low to medium traffic densities, showing some tolerance to human disturbance and taking advantage of resources provided by roads, such as food or perching sites (Meunier et al. 2000, Dean and Milton 2003). The most human tolerant species, such as kites, showed the largest increase in abundance. These opportunistic species show high tolerance to human disturbances, being able to benefit from anthropogenic resources (Blanco 1994, Meunier et al. 2000), using their high maneuverability and fast reaction to avoid threats (Blumstein et al. 2005). They can profit from food resources commonly found near roads, such as high prey density (Ruiz-Capillas et al. 2013b) or carcasses from roadkills (1974). The black kite was the only species that was not negatively affected by high traffic volumes, and also showed a strong selection for

roadkill hotspots. Roads and motorways are areas easy to spot and for a generalist predator they could provide food in a more predictable way than random movements along the landscape, as it is suggested by the use of road verges described for some generalist predators, such as stone martens (*Martes foina*), red foxes (*Vulpes vulpes*) or raccoons (*Procyon lotor*) (Frey and Conover 2006, Grilo et al. 2012, Ruiz-Capillas et al. 2013a).

Contrary to other studies, we did not find a positive effect of roads per se on buzzards (Meunier et al. 2000, Bautista et al. 2004). The abundance of these species near roads could anyhow increase if the availability of perching sites and prey were higher in these places than at random (Knight and Kawashima 1993, Palomino and Carrascal 2007). Also, the high frequency of zeros in our data could have influenced this result. However, it is unlikely that variables strictly related to roads, as the availability of carcasses, would attract these species because carrion is not a main part of their diet (del Hoyo et al. 1994).

The griffon vulture also increases it abundance near low traffic areas, although rapidly declined when traffic increased, similar to results of Bautista et al (2004). . Large species have greater alert distances and they need more time to initiate flight than smaller ones (Blumstein et al. 2005). Therefore, a constant flow of incoming vehicles will make it difficult for them to use a carcasses and thus they prefer areas with low or no traffic (Lambertucci et al. 2009, Zuberogoitia et al. 2010). Also, while our surveys were carried out there were no feeding stations for vultures in the study area and leaving carcasses from farms in the field is forbidden by European law (Donázar et al. 2009). Thus, finding food randomly distributed across the landscape might be a difficult task and, as it happens with other species, the proximity of roads may increase the likelihood of finding

a carcass (Monsarrat et al. 2013). Vultures have been suffering from a shortage of food due to the removal of livestock carcasses by sanitary measures (Donázar et al. 2009). This situation together with non-optimal flight conditions for long distance flight like those of cold days in winter (Margalida et al. 2010, Monsarrat et al. 2013), may have forced vultures to search for carrion in areas otherwise avoided, facing new risks that can deteriorate demographic parameters (Margalida et al. 2014). Under low food availability, vultures become more tolerant to taking risks (Zuberogoitia et al. 2010) in order to exploit more predictable resources (García-Heras et al. 2013, Margalida et al. 2013), which would explain their attraction to roadkill hotspots. In addition, close to these areas there might be abundant carrion from injured animals that moved outside the road, or roadkilled ones that were projected some meters away after the impact with the vehicle. Besides, roadkill hotspots also reflect areas with high abundance of the roadkilled species, mainly rabbits, and proximity to these areas will increase the chance of finding dead or ill individuals to feed on.

In the case of the booted eagle, its abundance was driven by the abundance of prey, with no effect of roads or motorways. This species is tolerant to human presence (Palomino and Carrascal 2007), but it is an active predator that obtains food from hunting instead of scavenging (del Hoyo et al. 1994). If no avoidance of roads occurs, we can expect to find more eagles near roads only at those points with large prey populations nearby.

Although we only used the traffic volume in our models, this predictor was highly correlated with other traffic characteristics that might also explain raptors response to roads, such as speed limit. Sampling plots located in control areas had no traffic, road plots presented low to medium traffic with speed limit of 90

kmh-1, and motorway plots had medium to high traffic and speed limit of 120 kmh-1. As a general trend, the species were tolerant to traffic to some degree and when high traffic volume increased they showed a negative response, accordingly with the expectation of traffic density affecting vertebrate use of roads (Bautista et al. 2004, Gunson et al. 2011), for which more tolerant species had higher thresholds. Raptors might perceive areas with more traffic as more dangerous places due to the higher speed limit (Legagneux and Ducatez 2013), the disturbance created by noise (McClure et al. 2013), and the continuous flow of vehicles may prevent them from feeding on the surface.

Furthermore, the abundance patterns observed might be influenced not only by the average daily traffic, but as also by the specific traffic volume during the activity hours of raptors, the type of vehicles using the infrastructure, or the speed limit (Forman et al. 2002, Legagneux and Ducatez 2013). Trucks could cause higher disturbance than smaller cars, especially if the peak traffic hours overlap with foraging activity of raptors. Further research is therefore needed to clarify the relationships of these variables in respect to raptor response.

4.4.2 Response to transport infrastructure across scales

The patterns of selection or avoidance of the infrastructure at the landscape scale were not reflected in the analyses of local infrastructure use for many species. When focusing only in the area around infrastructure, we detected an effect of traffic for two species, the red kite, that used the infrastructure above its availability, and the griffon vulture, that avoided the infrastructure. These patterns are parallel to those of the broad scale, and they point to strong positive selection by kites and strong avoidance by vultures of roads when traffic increases. Kites pro-

bably feed close to the infrastructure, while vultures probably feed on carcasses that are not too close to the asphalt surface (Lambertucci et al. 2009).

We did not detect any response for the other species, pointing to the possibility that the main habitat selection and therefore the response to roads happen at the broad scale (Rettie and Messier 2000). Thus, the response of raptors to traffic may be difficult to assess when analyzing only individuals close to the infrastructure, as some individuals might use roads even if the species in general prefers areas with low traffic (Basille et al. 2013). Also, individuals flying along the road also focus on the nearby terrain, and move away from the vertical of the asphalt surface for brief time periods to increase the amount of scanned surface for food (personal observation). Thus, punctual data on the location of birds might make difficult the detection of asphalt selection, even for individuals following the road. Besides, the lack of asphalt surface avoidance might be due to the low proportion that the roads occupy inside each observation plot (mean and SD of 18.5 ± 3.89 %), thus the statistical detection of significant negative effects becomes difficult. Maybe a larger dataset or more specific methods to follow the raptor movements with accuracy over time (e.g. high resolution telemetry) would help to better understand raptors responses to roads at a local scale.

4.4.3 Transport infrastructure and raptor conservation

As highlighted by our landscape level analyses, the effect of transport infrastructures was not restricted to the area above or adjacent to such infrastructures, but it extended into the landscape (Benítez-López et al. 2010) with two main effects: Infrastructures reduced the habitat available to species avoiding traffic and attracted opportunistic and tolerant species. Also, some species may be attracted to

roads by the presence of food, such as availability of prey or carrion, when there is few other sources in the landscape.

We should be cautious when interpreting the higher abundance of some species in certain areas affected by human activity. High abundances in risky areas could lead to negative effects on the population in the long term (Mumme et al. 2000, Delibes et al. 2001, Gomes et al. 2009). Collisions with cars are an important cause of mortality for birds (Møller et al. 2011), and raptors are more affected than other species (Dean and Milton 2003). Therefore, the availability of food can transform roads into population traps.. In addition, by increasing the presence and abundance of generalist or human-tolerant species, human-modified landscapes promote biotic and functional homogenization (Olden et al. 2004, McKinney 2006), at the cost of specialist species (Devictor et al. 2007), and making species more dependent on resource subsidies (McKinney 2006). Also, recent studies have shown that even for road selecting raptors, the stress caused by traffic can decrease their reproductive success (Strasser and Heath 2013).

Many raptor species show decreasing populations in recent years and are listed in the IUCN red list, including opportunistic species such as kites (IUCN 2013). Measures to reduce food availability near roads while ensuring the presence of natural food resources in the landscape should be implemented to reduce avian exposure to traffic and mortality. Long term population effect studies are needed to assess the indirect effects of roads on them. Finally, raptors are top predators and they may control ecosystem dynamics by top-down regulation of prey, with potential changes in the whole community structure when the raptor community is modified (see review on top-down regulation in Estes et al. 2011).

4.5 Acknowledgments

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4.6 Supplementary material

Table 4.4. Frequency of individuals of the species observed. During each season, we surveyed 20 of 240 data, after the two winters and two breeding seasons.

Species		Status[1]	CONTROL				
			B1	W1	B2	W2	Total
Red Kite	Milvus milvus	Resident	7	18	3	16	44
Black Kite	Milvus migrans	Breeding	24	0	20	0	44
Common/ Lesser Kestrels	Falco tinnunculus/ naumanni	Resident/ Breeding	6	3	8	3	20
Griffon Vulture	Gyps fulvus	Resident	2	6	1	7	16
Booted Eagle	Hieraaetus pennatus	Breeding	7	12	0	0	19
Common Buzzard	Buteo buteo	Resident	2	3	6	3	14
Cinereous Vulture	Aegypius monachus	Resident	1	2	7	3	13
Montagu's Harrier	Circus pygargus	Breeding	5	0	1	0	6
Hen Harrier	Circus cyaneus	Resident	0	1	0	0	1
Golden Eagle	Aquila chrysaetos	Resident	1	1	0	1	3
Short-toed Eagle	Circaetus gallicus	Breeding	0	0	1	0	1
Spanish Imperial Eagle	Aquila adalberti	Resident	0	1	0	0	1
Western Marsh-harrier	Circus aeruginosus	Resident	0	0	0	0	0
Peregrine Falcon	Falco peregrinus	Resident	1	0	2	0	3
Eurasian Sparrowhawk	Accipiter nisus	Resident	0	1	0	0	1
Eurasian Hobby	Falco subbuteo	Breeding	0	0	0	0	0
Long-legged Buzzard*	Buteo rufinus*	-	0	1	0	0	1
Total			56	49	49	33	187

1 Status described in Sanz-Zuasti and Velasco (2001).

B = Breeding season; W = Winter season.

* Rare species.

plots of each type (control, road, motorway), giving a total n of 80 per type, and a general total

ROAD					MOTORWAY					TOTAL
B1	**W1**	**B2**	**W2**	**Total**	**B1**	**W1**	**B2**	**W2**	**Total**	
10	28	8	40	86	12	28	8	28	76	206
40	0	36	0	76	37	0	29	0	66	186
14	4	5	3	26	6	6	5	8	25	71
3	9	7	3	22	7	14	4	2	27	65
3	18	0	0	21	8	15	0	0	23	63
4	7	2	9	22	5	7	3	8	23	59
2	3	3	6	14	6	6	6	2	20	47
1	0	4	0	5	1	0	1	0	2	13
0	3	0	1	4	0	2	0	0	2	7
0	0	0	1	1	0	0	0	1	1	5
0	0	0	0	0	2	0	2	0	4	5
0	2	1	0	3	0	0	0	0	0	4
2	0	0	0	2	1	1	0	0	2	4
0	0	1	0	1	0	0	0	0	0	4
0	0	0	0	0	0	0	1	0	1	2
1	0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	1
80	74	67	63	284	85	79	59	49	272	743

Table 4.5. Community analyses. Models explaining relative diversity, abundance and richness at Habitat structure, (ii) Food availability. Diversity models follow gaussian distribution. Abun-

Predictors	**Diversity**	
	AICc	**ΔAICc**
(0) Null model		
~ 1	356.8	32.2
(i) Habitat structure		
~ season + habitat + L. Dvill + adt^2	330.5	5.9
~ season + adt^2	324.6	0.0 *S
~ season + habitat	329.9	5.2
~ season + L.Dvill	326.8	2.2
(ii) Food availability		
~ season +L. HTrkill + L.MTrkill + L.rabbits + micros	333.2	8.6
~ season + L.HTrkill + L.MTrkill	329.2	4.5
~ season + L. rabbits + micros	329.8	5.2
(i) and (ii) Habitat + Food		
~ season + habitat + L. Dvill + adt^2 + L.HTrkill + L.MTrkill + L. rabbits + micros	338.3	13.7
~ season + L.HTrkill + L.MTrkill + L. rabbits + micros + adt^2	332.6	7.9
~ season + L.HTrkill + L.MTrkill + adt^2	328.9	4.3
~ season + L. rabbits + micros * adt^2	330.8	6.2
~ season + L. rabbits + micros + adt^2	328.2	3.6

Variables marked with "^2" were included in the analyses in their quadratic form (variable +

* Models within Δ ≤ 2 of the best model. When nested models are included in this subset, only

S Models selected for averaging.

1 Overdispersion value.

landscape scale. Models are presented within one of the tested hypotheses: (0) intercept only, (i) dance and richness follow poisson distributions.

Abundance			Richness		
Overdisp[1]	**AICc**	**ΔAICc**	**Overdisp[1]**	**AICc**	**ΔAICc**
1.58	1083.8	40.6	1.20	807.8	29.0
1.47	1052.8	9.5	1.07	786.2	7.4
1.44	1043.3	0.0 *S	1.06	778.8	0.0 *S
1.44	1052.3	9.1	1.08	784.0	5.3
1.42	1046.3	3.0	1.08	780.9	2.1
1.44	1052.9	9.7	1.09	786.9	8.1
1.43	1048.8	5.5	1.08	783.0	4.2
1.43	1049.1	5.8	1.08	783.5	4.7
1.50	1061.2	18.0	1.09	794.4	15.6
1.46	1051.2	8.0	1.08	786.3	7.5
1.45	1047.0	3.8	1.07	782.9	4.1
1.47	1046.2	2.9	1.07	783.8	5.0
1.45	1047.3	4.0	1.07	782.1	3.3

variable2). All models include the identity of the observation point as random factor (1|Pt.ID).

the model with lowest AICc is considered for further analyses.

Table 4.6. Species-specific analysis: red kite (*M. milvus*). Landscape foraging habitat selection models for red kite. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability, (iii) interaction with other species.

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	1.40	637.2	62.6	
(i) Habitat structure				
~ season + habitat + L.Dvill + adt^2	1.22	580.6	5.9	
~ season + adt^2	1.17	574.6	0.0	*S
~ season + habitat	1.17	584.1	9.5	
~ season + L.Dvill	1.16	574.7	0.1	*S
(ii) Food availability				
~ season + L.HTrkill + L.MTrkill + L.rabbits + micros	1.18	582.6	7.9	
~ season + L.HTrkill + L.MTrkill	1.15	580.9	6.3	
~ season + L.rabbits + micros	1.17	578.3	3.7	
(i) and (ii) Habitat + Food				
~ season + habitat + L.Dvill + adt^2 + L.HTrkill + L.MTrkill + L.rabbits + micros	1.23	587.5	12.8	
~ season + L.HTrkill + L.MTrkill + L.rabbits + micros + adt^2	1.20	580.6	6.0	
~ season + L.HTrkill + L.MTrkill + adt^2	1.19	578.0	3.4	
~ season + L.rabbits + micros * adt^2	1.20	580.6	5.9	
~ season + L.rabbits + micros + adt^2	1.19	577.3	2.6	
(iii) interaction with other species, habitat and food				
~ season + habitat + adt^2 + L.Dvill + migrans	1.22	582.6	8.0	
~ season + habitat + adt^2 + L.Dvill + pennatus	1.21	582.5	7.9	
~ season + L.HTrkill + L.MTrkill + L.rabbits + micros + migrans	1.19	584.5	9.8	
~ season + L.HTrkill + L.MTrkill + L.rabbits + micros + pennatus	1.17	583.9	9.3	
~ season + L.HTrkill + L.MTrkill + migrans	1.16	582.7	8.1	
~ season + L.HTrkill + L.MTrkill + pennatus	1.15	582.5	7.8	
~ season + L.rabbits + micros + migrans	1.18	580.2	5.6	

Predictors	Overdisp	AICc	ΔAICc	
~ season + L.rabbits + micros + pennatus	1.16	579.7	5.0	
~ season + adt^2 + migrans	1.18	576.6	1.9	*
~ season + adt^2 + pennatus	1.17	576.3	1.6	*
~ season + migrans	1.16	579.1	4.4	
~ season + pennatus	1.14	578.9	4.3	

All models follow poisson distribution and include the identity of the observation point as random factor (1|Pt.ID). Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within $\Delta \leq 2$ of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.7. Species level analysis: *Milvus migrans.* Landscape foraging habitat selection models for black kite. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability, (iii) Interaction with other species.

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	0.80	390.3	2.5	
(i) Habitat structure				
~ habitat + L.Dvill + adt^2	0.88	399.1	11.4	
~ adt^2	0.83	390.6	2.8	
~ habitat	0.84	397.1	9.4	
~ L.Dvill	0.81	390.1	2.3	
(ii) Food availability				
~ L.HTrkill^2 + L.MTrkill + L.rabbits + micros	0.90	390.7	2.9	
~ L.HTrkill^2 + L.MTrkill	0.87	387.8	0.0	*S
~ L.rabbits + micros	0.83	392.9	5.1	
(i) and (ii) Habitat + Food				
~ habitat + L.Dvill + adt^2+ L.HTrkill^2 + L.MTrkill + L.rabbits + micros	0.98	402.3	14.5	
~ L.HTrkill^2 + L.MTrkill + L.rabbits + micros + adt^2	0.91	393.2	5.4	
~ L.HTrkill^2 + L.MTrkill + adt^2	0.89	389.0	1.2	*S
~ L.rabbits + micros * adt^2	0.88	399.0	11.3	
~ L.rabbits + micros + adt^2	0.85	394.7	7.0	
(iii) interaction with other species, habitat and food				
~ habitat + adt^2 + L.Dvill + milvus	0.88	401.5	13.7	
~ habitat + adt^2 + L.Dvill + pennatus	0.89	401.5	13.7	
~ L.HTrkill^2 + L.MTrkill + L.rabbits + micros + milvus	0.91	392.9	5.1	
~ L.HTrkill^2 + L.MTrkill + L.rabbits + micros + pennatus	0.91	393.0	5.2	
~ L.HTrkill^2 + L.MTrkill + milvus	0.88	389.9	2.2	
~ L.HTrkill^2 + L.MTrkill + pennatus	0.89	389.9	2.1	

Predictors	Overdisp	AICc	ΔAICc
~ L.rabbits + micros + milvus	0.84	394.8	7.0
~ L.rabbits + micros + pennatus	0.83	395.0	7.3
~ adt^2 + milvus	0.84	392.7	5.0
~ adt^2 + pennatus	0.84	392.8	5.0
~ milvus	0.81	392.1	4.3
~ pennatus	0.81	392.4	4.6

All models follow poisson distribution and include the identity of the observation point as random factor (1|Pt.ID).

Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within $\Delta \leq 2$ of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.8. Species-specific analysis: booted eagle (*H. pennatus*). Landscape foraging habitat selection models for booted eagle. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability, (iii) interaction with other species.

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	0.98	243.2	5.1	
(i) Habitat structure				
~ habitat + L.Dvill^2 + adt^2	1.11	253.9	15.7	
~ adt^2	1.02	245.2	7.1	
~ habitat	1.03	249.7	11.5	
~ L.Dvill^2	1.00	244.5	6.3	
(ii) Food availability				
~ L.rabbits + micros	1.05	238.2	0.0	*S
(i) and (ii) Habitat + Food				
~ habitat + L.Dvill^2 + adt^2 + L.rabbits + micros	1.17	249.1	10.9	
~ L.rabbits + micros * adt^2	1.11	242.1	3.9	
~ L.rabbits + micros + adt^2	1.07	241.8	3.7	
(iii) interaction with other species, habitat and food				
~ habitat + adt^2 + L.Dvill^2 + milvus	1.12	256.0	17.8	
~ habitat + adt^2 + L.Dvill^2 + migrans	1.18	254.9	16.8	
~ L.rabbits + micros + milvus	1.07	240.1	1.9	*
~ L.rabbits + micros + migrans	1.08	240.1	1.9	*
~ adt^2 + milvus	1.03	247.2	9.1	
~ adt^2 + migrans	1.06	246.9	8.7	
~ milvus	0.99	245.0	6.9	
~ migrans	1.01	244.7	6.5	

All models follow poisson distribution and include the identity of the observation point as random factor (1|Pt.ID).

Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within $\Delta \leq 2$ of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.9. Species-specific analysis: common buzzard (*B. buteo*). Landscape foraging habitat selection models for common buzzard. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability, (iii) interaction with other species.

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	0.77	282.2	0.8	*S
(i) Habitat structure				
~ habitat + L.Dvill + adt^2	0.77	289.5	8.0	
~ adt^2	0.76	284.7	3.2	
~ habitat	0.77	284.7	3.3	
~ L.Dvill	0.77	283.4	1.9	*S
(ii) Food availability				
~ L.HTrkill + L.MTrkill + L.rabbits + micros	0.78	286.7	5.3	
~ L.HTrkill + L.MTrkill	0.77	286.1	4.7	
~ L.rabbits + micros	0.77	282.5	1.0	*
(i) and (ii) Habitat + Food				
~ habitat + L.Dvill + adt^2 + L.HTrkill + L.MTrkill + L.rabbits + micros	0.78	295.1	13.7	
~ L.HTrkill + L.MTrkill + L.rabbits + micros + adt^2	0.77	290.6	9.1	
~ L.HTrkill + L.MTrkill + adt^2	0.76	288.7	7.3	
~ L.rabbits + micros * adt^2	0.78	290.7	9.3	
~ L.rabbits + micros + adt^2	0.77	286.5	5.1	
(iii) interaction with other species, habitat and food				
~ habitat + adt^2 + L.Dvill + milvus	0.79	290.7	9.3	
~ habitat + adt^2 + L.Dvill + migrans	0.78	289.2	7.8	
~ habitat + adt^2 + L.Dvill + pennatus	0.77	291.7	10.2	
~ L.HTrkill + L.MTrkill + L.rabbits + micros + milvus	0.77	287.3	5.8	
~ L.HTrkill + L.MTrkill + L.rabbits + micros + migrans	0.77	285.8	4.3	
~ L.HTrkill + L.MTrkill + L.rabbits + micros + pennatus	0.77	288.8	7.3	
~ L.HTrkill + L.MTrkill + milvus	0.77	286.8	5.3	
~ L.HTrkill + L.MTrkill + migrans	0.77	285.5	4.1	
~ L.HTrkill + L.MTrkill + pennatus	0.77	288.1	6.6	

Predictors	Overdisp	AICc	ΔAICc	
~ L.rabbits + micros + milvus	0.76	283.0	1.6	*
~ L.rabbits + micros + migrans	0.75	281.6	0.1	*S
~ L.rabbits + micros + pennatus	0.76	284.5	3.0	
~ adt^2 + milvus	0.76	285.5	4.0	
~ adt^2 + migrans	0.75	283.4	1.9	*S
~ adt^2 + pennatus	0.76	286.7	5.3	
~ milvus	0.77	282.8	1.4	*S
~ migrans	0.77	281.4	0.0	*S
~ pennatus	0.77	284.1	2.7	

All models follow zero-inflated poisson distribution and include the identity of the observation point as random factor (1|Pt.ID).

Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within $\Delta \leq 2$ of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.10. Species-specific analysis: kestrels (*F. tinnunculus* and *F. naumanni*). Landscape foraging habitat selection models for kestrels. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	0.65	334.3	10.1	
(i) Habitat structure				
~ season + p.visib + habitat + L.Dvill + adt^2	0.65	332.4	8.2	
~ season + p.visib + adt^2	0.65	324.8	0.6	*S
~ season + p.visib + habitat	0.66	328.3	4.0	
~ season + p.visib + L.Dvill	0.66	324.2	0.0	*S
(ii) Food availability				
~ season + p.visib + micros	0.66	324.4	0.1	*S
(i) and (ii) Habitat + Food				
~ season + p.visib + habitat + L.Dvill + adt^2 + micros	0.65	334.6	10.4	
~ season + p.visib + micros * adt^2	0.65	328.0	3.8	
~ season + p.visib + micros + adt^2	0.65	326.9	2.7	

All models follow poisson distribution and include the identity of the observation point as random factor (1|Pt.ID).

Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within $\Delta \leq 2$ of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.11. Species-specific analysis: griffon vulture (*G. fulvus*). Landscape foraging habitat selection models for griffon vulture. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability.

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	0.981	303.966	0.000	*S
(i) Habitat structure				
~ habitat + adt^2	0.839	312.211	8.245	
~ adt^2	0.842	305.470	1.505	*S
~ habitat	0.896	311.149	7.183	
(ii) Food availability				
~ L.HTrkill + poly(L.MTrkill,2) + L.rabbits	0.900	306.689	2.723	
~ L.HTrkill + poly(L.MTrkill,2)	0.951	304.703	0.737	*S
~ L.rabbits	0.902	305.610	1.645	*S
(i) and (ii) Habitat + Food				
~ habitat + adt^2 + L.HTrkill + poly(L.MTrkill,2) + L.rabbits	0.746	316.461	12.495	
~ L.HTrkill + poly(L.MTrkill,2) + L.rabbits + adt^2	0.760	308.223	4.257	
~ L.HTrkill + poly(L.MTrkill,2) + adt^2	0.847	306.783	2.818	
~ L.rabbits * adt^2	1.054	306.801	2.836	
~ L.rabbits + adt^2	0.747	306.879	2.913	

All models follow zero-inflated poisson distribution and include the identity of the observation point as random factor (1|Pt.ID).

Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within $\Delta \leq 2$ of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.12. Species-specific analysis: cinereous vulture (*A. monachus*). Landscape foraging habitat selection models for cinereous vulture. Models are presented within one of the tested hypotheses: (0) intercept only, (i) Habitat structure, (ii) Food availability.

Predictors	Overdisp	AICc	ΔAICc	
(0) Null model				
~ 1	0.947	243.798	3.899	
(i) Habitat structure				
~ habitat + adt^2	0.780	249.287	9.388	
~ adt^2	0.900	245.718	5.820	
~ habitat	0.789	245.573	5.674	
(ii) Food availability				
~ L.HTrkill + L.MTrkill^2 + L.rabbits^2	0.731	240.077	0.179	*S
~ L.HTrkill + L.MTrkill^2	0.800	239.899	0.000	*S
~ L.rabbits^2	0.898	245.048	5.150	
(i) and (ii) Habitat + Food				
~ habitat + adt^2 + L.HTrkill + L.MTrkill^2 + L.rabbits^2	0.698	248.593	8.694	
~ L.HTrkill + L.MTrkill^2 + L.rabbits^2 + adt^2	0.739	243.909	4.010	
~ L.HTrkill + L.MTrkill^2 + adt^2	0.803	242.915	3.017	
~ L.rabbits^2 * adt^2	0.823	254.450	14.551	
~ L.rabbits^2 + adt^2	0.836	246.517	6.618	

All models follow zero-inflated poisson distribution and include the identity of the observation point as random factor (1|Pt.ID).

Variables marked with "^2" were included in the analyses in their quadratic form (variable + variable2).

* Models within Δ ≤ 2 of the best model. When nested models are included in this subset, only the model with lowest AICc is considered for further analyses.

S Models selected for averaging.

Table 4.13. Coefficients of community level models. Only variables in the selected models within 2 points of AICc were used. Explanatory variables are grouped by the hypothesis they belong: (i) Habitat structure. Values for the intercept and controlling variables are also included although they are not considered informative parameters for these analyses. For a definition of the variables see table 4.1.

Variable	Diversity	Abundance	Richness
(Intercept)	0.713	1.263	0.853
Controlling variables			
Season [Winter]	-0.361	-0.473	-0.528
(i) Presence of roads or motorways			
ADT	0.714	1.998	1.216
ADT2	-0.841	-1.260	-1.090

Table 4.14. Averaged coefficients of species-specific models. Only variables in the selected models within 2 points of AICc were used. Variables with an averaged coefficient close to zero (≤|0.01|) are marked as NE (no effect). For a definition of the variables see table 4.4.

Variable	Red kite *M. milvus*	Black kite *M. migrans*	Booted eagle *H. pennatus*	Common buzzard *B. buteo*	Kestrels	Griffon vulture *G. fulvus*	Cinereous vulture *A. monachus*
(Intercept)	-0.170	1.013	-1.277	-0.486	-8.142	-0.183	0.601
Controlling variables							
Season [Winter]	1.193				-0.489		
Visib					7.569		
(i) Habitat struture							
ADT	1.301	0.911		0.101	0.777	0.215	
ADT2	-0.785	0.279		-0.199	-0.619	-0.762	
L.Dvill	-0.108			0.021	-0.041		
(ii) Food availability: Natural prey and anthropogenic food resources (roadkill)							
Rabbits			0.212	-0.021		-0.010	-3.450
Rabbits2							-5.132
Voles			NE	NE	NE		
HT.Rkill		-0.034				0.012	-0.157
HT.Rkill2		3.379					
MT. Rkill		-0.082				-0.299	-9.300
MT. Rkill2						-2.098	-6.971
(iii) Interaction with other species							
Milvus				0.018			
Migrans				-0.128			0.094

Table 4.16. Model coefficients for raptor response to asphalt surface within the sampling plots.

	adt	**adt^2**	**Season [Summer]c**	**Season [Winter]c**	**Time [Morning]c**	**Time [Afternoon]c**
M .milvus	6.00 ± 2.21**	-0.48 ± 1.97	1.38 ± 0.29***	0.53 ± 0.21*		
M. migrans	2.90 ± 2.60	0.03 ± 2.28			0.70 ± 0.27*	2.49 ± 0.36***
H. pennatus	-2.16 ± 3.72	-3.32 ± 2.96				
B. buteo	-2.90 ± 5.80	3.76 ± 5.14	1.33 ± 1.39	-2.07 ± 1.57		
F. tinnunculus + *F. naumanii*	0.36 ± 0.98	-1.61 ± 1.32	-5.31 ± 4.71	0.51 ± 3.74		
G. fulvus	-11.07 ± 3.66**	-9.66 ± 2.46***				
A. monachus	7.84 ± 6.19	-0.56 ± 4.46				

Value of the coefficient ± SD of explanatory variables in the analysis of asphalt cells.

Significance levels: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

adt: Average daily traffic.

c Controlling variable.

Chapter 5

Cascading effect in motorway verges from prey abundance to carnivore mortality

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Abstract

Anthropogenic habitat modification can affect the balance of predator-prey relationships. We study a system composed of European rabbits and a community of mesocarnivores in an area disturbed by motorways, to study whether the presence of abundant prey close to motorways cascades into predator mortality. We estimated indexes of rabbit and carnivore relative abundance/use by surveying scats in 1 km transects, and a roadkill index in motorway stretches parallel to the transects. For analyses, we considered four carnivore groupings: the whole carnivore community, mustelids, red fox, and *Felis* spp. When considering the whole carnivore community, we found a tendency towards higher abundance in areas near motorways compared to control sites. Furthermore, motorway stretches with higher rabbit abundance were associated with increased carnivore abundance that resulted in higher carnivore mortality by roadkill. When analyzed separately, mustelids were more likely to be present and fall victim of roadkill in areas of high rabbit abundance compared to red foxes, probably because the latter is more reliant on this food source and can use alternatives such as carrion or garbage. We conclude that prey populations on motorway verges may attract carnivores, with potentially fatal consequences, and thus should be managed carefully to assure conservation of wildlife species.

5.1 Introduction

Wildlife populations respond to anthropogenic disturbance in multiple ways. While some species avoid disturbance and suffer reduced densities (Saito and Koike 2013), others profit on the new conditions and thrive in areas potentially rich in resources (Fedriani et al. 2001, Kolowski and Holekamp 2008, Bino et al. 2010, Dellinger et al. 2013). Changes in habitat conditions, and the associated effects on species, can also lead to changes in the interspecific relationships, including shifts in predator-prey dynamics (Rodewald et al. 2011, Newsome et al. 2014).

Roads are a common and widespread case of human disturbance, which apart from altering habitat in different ways, also fragment available habitat and lead to direct mortality of fauna (Trombulak and Frissell 2000, Forman et al. 2003). Roads have multiple effects on wildlife species, from negative to positive. Among the described positive effects on wild communities, roads can provide new habitat in the strips of terrain next to the pavement known as road verges. Some small mammals thrive in them and reach dense populations there (Bellamy et al. 2000, Ruiz-Capillas et al. 2013b). In fragmented landscapes, carnivore habitat use is related to the availability of food resources (Mortelliti and Boitani 2008) and it has been suggested that small mammal populations in road verges, together with other anthropogenic resources such as roadkills and garbage, could attract carnivores to the proximity of roads (Barrientos and Bolonio 2009, Grilo et al. 2012).

Individuals located near roads are exposed to increased mortality risk by vehicle collision (Forman et al. 2003). Usually, prey species can compensate for

this additional mortality thanks to their high reproductive rates. However, carnivores typically have lower reproductive rates and an increase in their mortality may negatively affect their population viability in the long term (Rytwinski and Fahrig 2012). Differential mortality and tolerance to human disturbance may also affect the composition or abundance of individual species within the carnivore community. Thus, roads can act as demographic traps with major conservation implications, since only species that can avoid oncoming traffic will persist in the long run (Jaeger et al. 2005, Rytwinski and Fahrig 2012).

In the Iberian Peninsula some road verges sustain large populations of prey, such as Apodemus sylvaticus and Mus spretus mice (Sabino-Marques and Mira 2011, Ascensão et al. 2012, Ruiz-Capillas et al. 2013b). European rabbits (*Oryctolagus cuniculus*) are also common in road verges in some habitats (Barrientos (Bautista et al. 2004, Barrientos and Bolonio 2009, but see Planillo and Malo 2013). Rabbits are native to the Iberian Peninsula and a key prey species in Mediterranean ecosystem (Delibes-Mateos et al. 2008b). In addition to rabbit specialists, such as the Iberian lynx (Delibes 1980), many other carnivores, such as wildcats (*Felis silvestris*) and red foxes (*Vulpes vulpes*), prefer this species when available (Carvalho and Gomes 2001, Malo et al. 2004a, Barrientos and Virgos 2006, Delibes-Mateos et al. 2008a). Therefore, if rabbit populations establish in road verges, they may attract carnivores and thus create a cascading effect that could lead to carnivore mortality in such areas, with important consequences for conservation and the management of road impacts.

In this study, we focus on a community composed of rabbits, as the main prey, and several carnivore species in a typical Mediterranean landscape. We analyze the carnivore response to variability in rabbit abundance in motorway

verges by specifically testing three hypotheses: 1.Carnivores prefer areas near motorways over control areas due to high abundance of prey and other resources. 2. Areas with higher prey abundance near motorways are characterized by a higher frequency of carnivore traffic casualties than areas with fewer preys. 3. We further predict that roadkills of specialist carnivores will be more tightly associated to areas with high prey abundance than roadkills of generalist carnivores, since the latter will also use other resources along roads, such as garbage or carrion, and they will be less concentrated in specific prey-rich areas.

5.2 Material and Methods

5.2.1 Study Area

The study was carried out in a rural area of central Spain located in Ávila and Segovia provinces, with a human population density of 25.5 inhabitants per km2. The area is situated in the Spanish Northern Plateau and has a Continental Mediterranean climate, characterized by cold winters (mean temperature of 5º C), dry summers (17º C), and average annual precipitation of 408-573 mm (Ninyerola et al. 2005). Natural vegetation is dominated by sclerophyll shrubs, with some Holm oak forests (Quercus ilex), and riparian forest along rivers. Traditional land uses include cattle grazing pastures with open woodland ("dehesas") and some extensive croplands for non-irrigated cereals. The study area includes three areas of high conservation value included in the European Natura 2000 network that cover a surface of more than 500 km2.

Ten of 18 wild carnivore mammals present in Spain can be found in our study area, three of them being listed as Near Threatened in UICN red list for

Spain (Supplementary Table 5.1, Palomo et al. 2007). Feral cats and dogs are also common in the area.

For this study, we selected three motorways: AP-6, AP-51 and AP-61 (Fig. 5.1A). AP-61 and AP-51 have medium traffic volumes (6472 and 7782 vehicles a day, respectively), and AP-6 has a high traffic volume (28 684 vehicles a day). All three motorways have a perimeter fence, effective only against ungulates and humans but permeable to smaller species.

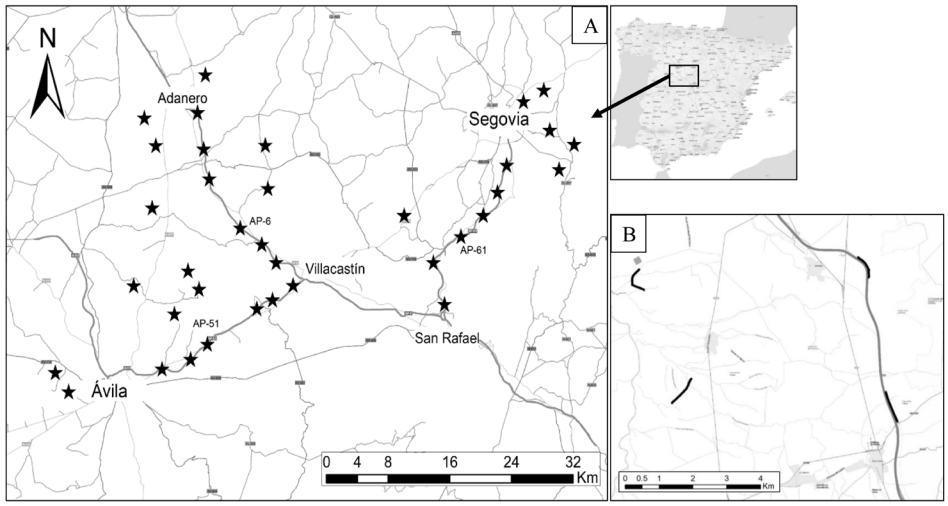

Figure 5.1. Study Area. A. Location of the study area in Spain and that of 1 km transects within it. Transects that appear away from motorways were considered control transects and the rest are the motorway ones. B. Enlargement of an area showing with detail two motorway transects and two control transects.

5.2.2 Data collection

A. Rabbit and Carnivore relative abundances

To estimate species relative abundance/use, we established 36 linear transects within the study area, 18 along motorways (6 in each motorway) and 18 in control zones (Fig. 5.1A). Transects were 1 km long, and control zones were at least 4 km away from motorways, as the home ranges of the common carnivore species are about 2.5 to 5.8 km^2 (Rosalino et al. 2004, Santos-Reis et al. 2005, Rondinini et al. 2006), making it very unlike that one individual would move as far as 4 km in linear distance. Control transects were located along rural dirt roads with very low traffic at 6.1 ± 1.3 km (mean ± sd) from the closest motorway, and they were randomly located within areas of similar habitat to the motorway transects (see below, Fig. 5.1B). Motorway transects were located parallel to motorways, as close as possible to the road (52 ± 21.9 m from motorways), randomly located on either side of the motorway, and usually along the perimeter fence. In our study area, along this fence there often dirt roads very similar to those in control sites that are barely used. The relative abundance/use of carnivores next to motorways was surveyed using these dirt roads and parallel to the fence. To avoid potential bias due to detectability or habitat (Gompper et al. 2006), sampling transects were located in similar habitats both in motorway and control zones, controlling for similar structure vegetation in both situations (e.g. for each motorway transect with shrub vegetation, there was a control transect with the same vegetation structure). We surveyed transects for two consecutive years, once in spring 2011 and once in spring 2012.

For each transect, we obtained a measure of relative abundance/use for rabbits and carnivores based on fecal counts. This is a widely used technique for

broad scale studies, as it provides comparable data without the necessity of capturing the animals (Gompper et al. 2006). Although the relative abundance cannot be directly converted into a number of individuals, it can be used to differentiate areas with higher or lower abundances provided that it is estimated under the same protocol (Cavallini 1994, Wilson and Delahay 2001, Fernandez-De-Simon et al. 2011a). Also, relative abundance based on fecal counts is a good measure of the intensity of use of a given area (Piñeiro and Barja 2015). For simplicity, in the text we use the expression rabbit or carnivore abundance when referring to our measures of relative abundance/use.

To obtain a measure of rabbit relative abundance, or rabbit index, we evenly distributed ten plots of 0.5 m^2 along each transect and counted the rabbit pellets within each plot, avoiding latrines to prevent bias (Fernandez-De-Simon et al. 2011a). The sum of all rabbit pellets found in each transect was used as the index value.

We estimated carnivore relative abundance, or carnivore index, as the number of scats detected in each transect. Scat density can be used to estimate carnivore densities over large spatial scales (Webbon et al. 2004), thus making it a good index to compare abundances. Surveys were performed during spring, when vegetation cover is still limited, and thus avoiding the high summer temperatures that cause rapid degradation and winter snow which covers the scats (Heinemeyer et al. 2008). In both years, the same two observers walked along transects and recorded all carnivore scats. The sum of scats detected on each transect was used as an index of carnivore abundance (Long et al. 2008). This index also serves as a measure of carnivore activity since those zones more used for hunting will be marked more intensively (Piñeiro and Barja 2015).

Each scat was assigned to a species based on morphological characteristics and following a conservative approach. When the identification was not clear, or there was no consensus between the two observers, the scat was classed as "unidentified carnivore". We detected all the species present in the study area (Table S5.1), excluding American mink, European otter and Common genet.

B. Carnivore roadkills

We estimated a carnivore roadkill index for the 1 km stretch of motorway closest to each motorway transect. Data on roadkilled carnivores were obtained from a database of carnivore carcasses and a complementary monthly survey by vehicle. The database was provided by the company responsible for the management of the motorways and it contained all the recorded casualties over 5 years, from 2007 to 2011. Motorway workers travel the motorways two or three times a day and record any carcass found in the traffic lanes that is big enough to be considered a safety hazard. Usually, this means that they record and collect animals that are the size of a stone marten or larger. The complementary survey by vehicle was carried out once a month during 2010 and 2011 with a car droved at low speed (30 kph), with a driver and a dedicated observer recording the location and species of all carcasses, complementing the database with carcasses found on the verges and those of smaller size. As our motorway stretches were only 1 km long and carcasses have low detectability and may be quickly removed by scavengers (Santos et al. 2011), for analytical purposes we computed the roadkill index as the sum of all the carnivore carcasses found over the five years.

5.2.3 Data Analyses

For the analyses, we grouped the carnivore taxa into five different species groups: Whole carnivore community (all data pooled, including undetermined scats), Red fox, Mustelids (all species from Mustelidae family, see Table S5.1, although stone martens comprised more than 50%), Felis spp. (feral and wild cats) and Canis spp. (feral dogs and wolves). We did DNA analysis of a subsample of fresh scats to assess the accuracy of the morphological determination (Long et al. 2011), following a protocol specifically designed for Iberian Peninsula carnivores (Fernandes et al. 2008). In our subsample, the DNA results for red foxes were consistent with morphological identification (100% accuracy), but for stone martens some inconsistency with field identification was detected, pointing to other mustelids and also foxes, with less than 50% accuracy at species level, as reported by other studies (Davison et al. 2002). In addition, carcass identification from roadkilled carnivores can be difficult to identify and similar species might be mistaken by motorway workers (for example, different mustelid species, or feral and wildcats that are not well preserved). Thus, we decided to pool together similar species to minimize the potential errors.

A. Carnivore response to rabbit abundance

To analyze carnivores' response to rabbits and the presence of the motorways, we used data of relative abundances from the whole study area, comparing control sites and motorways. For these analyses we followed two complementary approaches. First, we analyzed data from the whole carnivore community, and then we run specific models for the species groups described above. Because of

the low number of transects (n = 5) for Canis spp., this groups was not analyzed individually.

We first analyzed differences in the rabbit abundance index between motorway and control transects with liner mixed models (LMM), after log-transformation of the rabbit index. The response of carnivores to rabbits and proximity of motorways was analyzed with generalized linear mixed models (GLMMs), with a Poisson error structure and log link. In both analyses, the identity of the transect was included as a random factor. For the GLMMs, the initial models included four explanatory variables: the year (to control for temporal effects), rabbit abundance index (as a measure of prey availability), location of transect (motorway, control), and the interaction between rabbit abundance index and location of transect. When the interaction was found to be not significant using log-likelihood tests, it was removed from the model to obtain more reliable coefficients for the rest of explanatory variables (Zuur et al. 2009). Residuals and the dispersion parameter of the Poisson models were checked for model validation (Zuur et al. 2009).

In the results section we only present the results of the explanatory variables location of the transect and rabbit abundance index, since the variable year was used to control for temporal pseudoreplication, and thus it is not of interest in itself.

B. Relationship between rabbits, carnivores and roadkills on motorways

Focusing only on the motorway transects, we further investigated whether the abundance of rabbits had a cascading effect on carnivores, increasing their abun-

dance and correspondingly their mortality by roadkill. As we only had one value of roadkill for each motorway stretch (see above), we computed the mean rabbit abundance and the sum of carnivore abundance index obtained during the two surveys for each transect to make the data comparable.

For the whole carnivore community, the cascading effect was tested by path analysis, a modeling technique within the broader approach of Structural Equation Modelling (SEM, Sokal and Rohlf 2012). Path analysis allows testing for linear relationships between variables, with the advantage of including indirect effects mediated by a third variable or mediator (Kline 2005, Hoyle 2012). In our case, the mediator variable was carnivore abundance, following the hypothesis that rabbit abundance affects carnivore mortality as a consequence of an increase in carnivore abundance in motorway verges. Due to non-normality of variables, we used the Satorra-Bentler robust estimators, recommended for small sample sizes (Kline 2005, Hoyle 2012). The correct global adjustment of the SEM analysis was evaluated by several recommended indexes (Schermelleh-Engel et al. 2003, Garrido et al. 2005, Hoyle 2012): Joreskog GFI, RMSEA, TLI, and a corrected goodness-of-fit test between the observed and expected covariance matrices.

For the individual analyses of species groups, the datasets for red fox and mustelids were not large enough to obtain reliable results in the path analyses. Instead, we decided to use a more conservative approach and tested the partial responses of each species on rabbit abundance and their roadkills by generalized linear models (GLMs). The GLMs had a Poisson error structure and a log link, and models assumptions were checked by inspecting the residuals (Zuur et al. 2009).

Finally, due to the low observed abundances of Felis spp., this species group

was analyzed with Monte Carlo chi-squared tests for small sample size. We classified data in low versus high values for the variables involved (≤ 11 vs. ≥ 54 rabbit pellets; 0-1 vs. ≥ 2 scats; 0 vs. ≥ 1 roadkills), and we compared expected and observed frequencies. We run 10,000 Monte Carlo randomizations to compute probabilities.

All analyses were done in R 3.0.3 (R Core Team 2014). We used lme4 package package for GLMMs (Bates et al. 2014), and lavaan package for path analyses (Rosseel 2012). All results are presented as mean ± standard error, unless otherwise indicated.

5.3 Results

Average rabbit abundance index was 148.1 ± 29.4 pellets (range 0 - 597) in motorways and 52.8 ± 11.3 pellets (range 0 - 303) in control transects. Although some motorway transects had the highest values of the index, the LMM of the log-transformed data showed not significant differences between control and motorway transects ($F = 0.53$, $df = 1, 34$, $p = 0.473$).

5.3.1 Carnivore response to rabbit abundance and transect location

In the analysis of the whole study area, we found 868 carnivore scats, in the following proportions: 45% red fox, 27% mustelids, 3% Felis spp., 1% Canis spp., and 24% unidentified carnivores. The distribution of the raw data in each transect type follows the same pattern for almost all the species groups, with higher mean values in motorways than in control sites (Fig. 5.2).

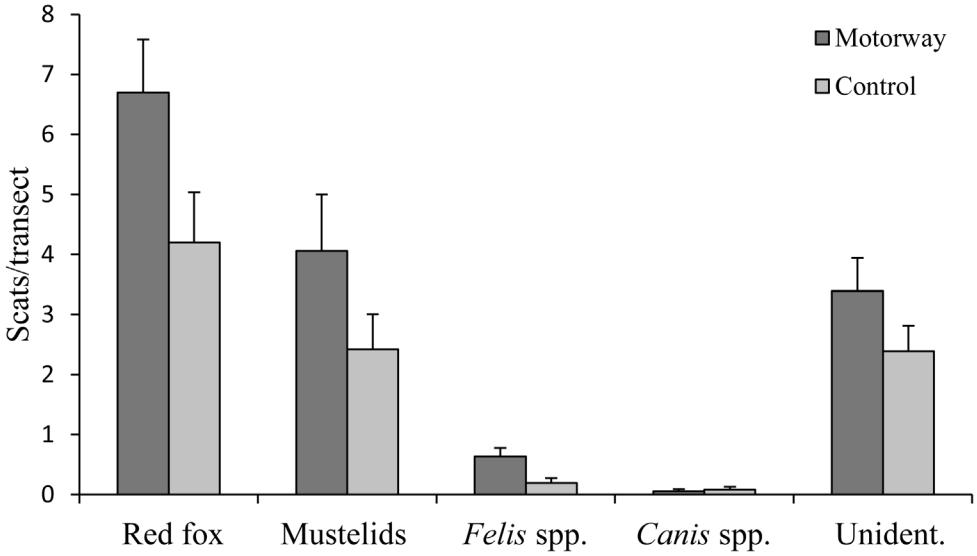

Figure 5.2. Carnivore relative abundance. Carnivore scats detected for each species group in motorway and control transects (mean + SE). Mustelids category includes all species from Mustelidae family present in the study area, except for American mink and European otter (see Supplementary Table 5.1).

The GLMMs models showed effects of both explanatory variables, location of the transect and rabbit abundance index, that depended on the group being analyzed. The global carnivore community index was significantly higher in the proximity of a motorway (βmotorway = 0.531 ± 0.241, p = 0.028), and marginally higher with rabbit abundance (βrabbit = 0.086 ± 0.050, p = 0.089). The red fox index was significantly higher near motorways βmotorway = 0.521 ± 0.241, p = 0.031) but showed no effect of rabbit abundance (βrabbit = 0.073 ± 0.055, p = 0.186). The mustelid index showed no detectable effect of proximity of a motorway or rabbit abundance (βmotorway = 0.352 ± 0.279, p = 0.207; βrabbit = 0.008 ± 0.066, p = 0.905). And finally, the Felis spp. index was significantly higher near motorways (βmotorway = 9.128 ± 3.742, p = 0.015) and at locations with higher rabbit abundance (βrabbit = 2.089 ± 0.737, p = 0.005). In summary,

the location of the transect was significant for three out of four response variables, and always pointed to higher values of abundance/use intensity near motorways. The rabbit abundance index showed a positive relations with all measures of carnivore abundance, but it was significant in only one case. As a result of both these effects, it seems that for the same values of rabbit abundance, carnivore abundance tends to be higher in motorway transects than control sites (Fig. 5.3). We found no interaction between location of transects and carnivore abundance, except for Felis spp. (βmotorway*rabbit: 1.77; p: 0.018), that showed a stronger relationship with the prey abundance in control sites (Fig. 5.3).

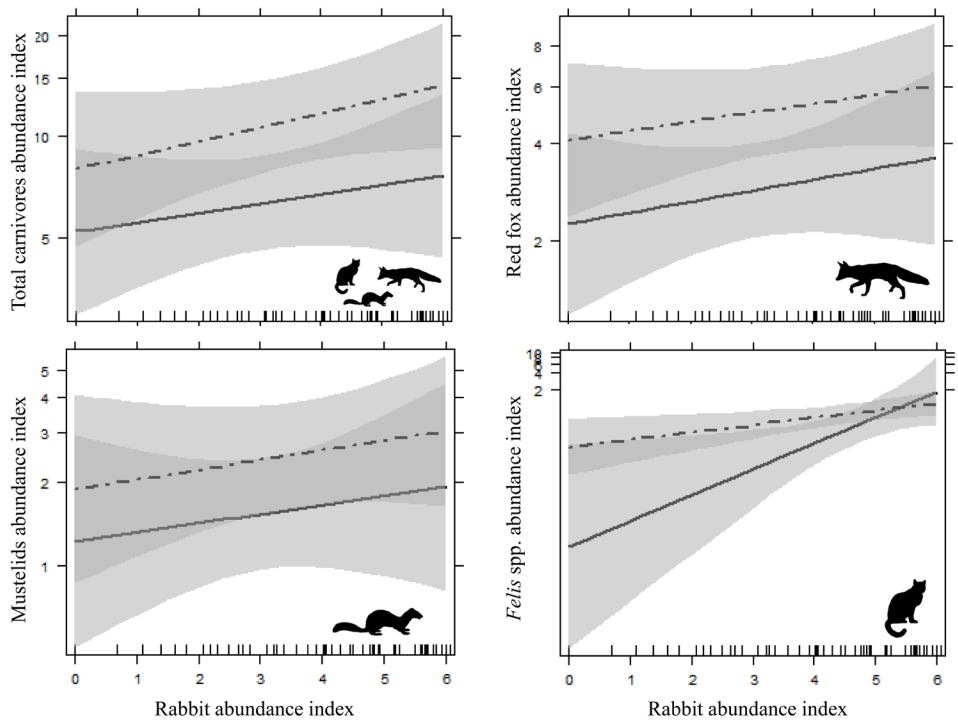

Figure 5.3. Carnivore response to rabbit abundance in motorway and control sites. Continuous lines represent control sites and dashed lines, motorways. Shadow areas represent confidence intervals, with darker areas where they overlap. Notice that y-axes are at different scales.

5.3.2 Cascading effects: Rabbit abundance - Carnivore abundance - Road-kills.

Focusing only on motorways, we found a total of 86 carnivore casualties in our 18 motorway stretches (57% red fox, 15% mustelids, 6% Felis spp., 20% Canis spp., and 2% unidentified carnivores). In the analyses for the global carnivore community, the overall model was significant and roadkills were positively and significantly associated with rabbit abundance through increased carnivore abundance, although the partial regression of carnivores on rabbit abundance was only marginally significant (Fig. 5.4A). As coefficients in this model are standardized, they can be compared. The partial effect of carnivore abundance on carnivore roadkills was higher ($\beta = 1.07$) than the effect of rabbit abundance on carnivore abundance ($\beta = 0.31$). The goodness-of-fit tests of the SEMs indicated that this model described well the relationships between rabbit abundance, carnivore abundance and carnivore roadkills ($\chi^2 = 0.814$, df = 1, $p = 0.367$; Joreskog GFI = 0.992, RMSEA = 0.000, TLI = 1.051).

In the species groups analyses, all carnivore relative abundances increased with rabbit relative abundance in transects next to motorway, as it is shown by the positive coefficients and a higher presence of Felis spp. in transects with high rabbit abundance than expected. The relationship was significant for Felis spp. ($\chi^2 = 7.20$, $p = 0.013$) and marginally significant for mustelids ($\beta = 0.08$, $p = 0.074$) (Fig. 5.4B). In addition, fox and mustelid roadkills were positively and significantly related to their abundances (Fox: $\beta = 0.05$, $p = 0.019$; Mustelid: $\beta = 0.04$, $p = 0.029$). There was no relationship between Felis spp. roadkills and Felis spp. relative abundance ($\chi^2 = 0.16$, $p = 0.999$) (Fig. 5.4B), although we only had four cat roadkill events, being very difficult to detect an effect. Three out of this four

roadkills occurred next to transects with Felis spp. scats.

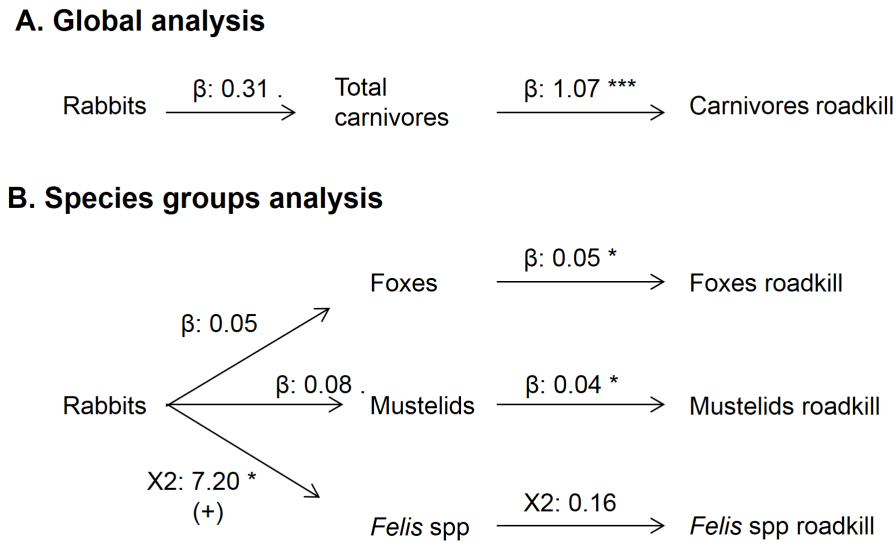

Figure 5.4. Effects of rabbit abundance in carnivore roadkills. A. Path coefficients of the structural equation model for global data showing a cascading effect from rabbit abundance to carnivore roadkill by means of increased carnivore abundance. B. Graphical representation of the relationships between rabbit relative abundance, carnivore relative abundance and carnivore roadkills. Models for species groups were built by GLMs for Foxes and Mustelids and and $\chi 2$ for Felis spp. In the case of the $\chi 2$, the sign "+" is included to show a positive correlation between the variables. Significant and marginally significant results are marked as: $p < 0.1$, * $p < 0.05$, *** $p < 0.001$.

5.4 Discussion

In accordance to our predictions, we found that carnivore abundance tends to increase in transects along motorways compared to control sites. Furthermore, focusing specifically on areas near motorways and analyzing the whole carnivore community, our results point to a cascading effect of prey abundance in verges leading to carnivore mortality, mediated by an increase in carnivore abundance.

It is important to note here that the interpretation that follows would be parallel if data were handled as use indexes: the more frequently that carnivores visit sites with abundant prey close to the motorway, the higher the roadkill risk they face.

When comparing control and motorway transects, the response of carnivores to rabbit abundance was similar across the whole area, and carnivores tended to be more abundant near motorways for the same number of prey, with the exception of Felis spp. (Fig. 5.3). Similar patterns of higher predator abundance near motorways have been already observed for some species in the Iberian Peninsula (Ruiz-Capillas et al. 2013a, Planillo et al. 2015). While under certain circumstances carnivores avoid roads to a degree (Jedrzejewski et al. 2004, Riley 2006, Basille et al. 2013), some species have shown a preference for feeding near linear infrastructures, even in the absence of higher prey abundance (James and Stuart-Smith 2000). This behavior may be partially explained if prey next to areas with high disturbance levels, such as motorways, are less aware of predators, thus making them easier to hunt (Barbosa and Castellanos 2005, Chan et al. 2010). We did not find a statistical difference in rabbit abundance between motorways and control sites. Motorway verges can reach very high values of rabbit abundance in specific spots, and also other prey species not measured in this study may be more abundant in verges (Ruiz-Capillas et al. 2013b), which may cause the attraction of carnivores to motorways. Roads also provide garbage used by opportunistic species and carrion for scavengers (Clevenger and Wierzchowski 2006). The higher availability of resources may thus compensate for disturbance and make carnivore more prone to wander close to roads, as resource availability is one of the main drivers of habitat use for this group (Barbosa and Castellanos 2005, Mortelliti and Boitani 2008, Boitani and Powell 2012). In addition to these explanations, carnivores may use roads as home range boundaries and thus visit

them often to mark and prevent other individuals from entering their territory (Riley 2006).

Anyhow, it seems that the abundance of resources attracts carnivore species to the proximity of traffic to some degree, and as a consequence higher mortality by roadkill will happen in those motorway stretches that are more attractive to carnivores. Since one key attractant in this case is the abundance of rabbits and it varies along motorway verges, carnivore roadkills will concentrate where rabbits are more abundant in verges.

Regarding our third prediction, that roadkills of specialist carnivores would be more closely related to areas of high prey abundance than in the case of generalists, the results are unconclusive. Mustelids represent the specialist species in our study area, as they select live prey and especially rabbits when available (McDonald et al. 2000, Herr et al. 2009, Bateman and Fleming 2012). On the other hand, red fox is a rather opportunistic species, highly adaptable, and it is not much influenced by live prey (Bateman and Fleming 2012). The analysis of data collected in motorway transects shows that the effect of prey is somewhat noticeable in carnivore abundance for specialist species, but not so for the opportunistic ones: prey abundance in motorway transects was positively (though marginally) related to mustelid abundance, but not to red fox abundance. This result suggests but does not prove that spots of high rabbit abundance near motorway could create potential roadkill hotspots for mustelids at a landscape scale, as suggested for polecats (Mustela putorius) at a regional scale (Barrientos and Bolonio 2009).

The lack of significance at our scale between prey abundance and the opportunistic carnivore, red fox, is in line with our hypothesis and probably caused by the availability of other resources along motorway verges. When anthropogenic

resources are available, foxes prefer them instead of natural prey and rely heavily on food from human origin (Baker et al. 2007, Bino et al. 2010). Even if not related to rabbit abundance, the relationship between fox abundance and roadkills is not surprising. The use of anthropogenic resources in disturbed areas by opportunistic carnivores, such as foxes, coyotes, raccoons or bears, is often associated with an increased mortality (Gosselink et al. 2007, Beckmann and Lackey 2008, Bateman and Fleming 2012). The high proportion of foxes in the global dataset of carnivores can also explain the lack of a statistically stronger relationship of them with prey abundance.

When we analyzed data at the landscape scale, comparing control transects with motorway transects, mustelid abundance was not significantly higher near motorways nor related to rabbit abundance, suggesting that the effect of prey abundance on mustelid roadkill may be only local, perhaps restricted to areas with very high prey abundance. In the light of our results, we cannot confirm the effect of prey abundance on specialist carnivores and more detailed studies are needed.

Roadkills are among the main causes of human-induced mortality for several carnivore species, including foxes, black bears, Iberian lynx, panthers and badgers (Maehr et al. 1991, Ferreras et al. 1992, Takeuchi and Koganezawa 1994, Brandenburg 1996, Clarke et al. 1998, Snow et al. 2012), and compromise the success of some reintroduction programs (Kramer-Schadt et al. 2004). We believe that the detection of a trend in carnivores to approach anthropogenic risky areas should be considered carefully from a conservation point of view. Although a high abundance of a species in a human-made habitat might seem positive, if it is associated to high mortality rates, it might lead to a population sink (Delibes

et al. 2001). Ecological traps have been found primarily in habitats modified by human activities as those related to roads (Battin 2004, Nielsen et al. 2006, Beckmann and Lackey 2008, Falcucci et al. 2009), which also contribute to the general homogenization of ecosystems (McKinney 2006). Thus, understanding wildlife responses to human-modified landscapes and studies on long term population effects are necessary in order to make informed management decisions.

The abundance of prey in road verges is a phenomenon that should be treated carefully. The design and management of verges could be a key feature for conservation of prey and predator populations. Our results support the recommendation that in landscapes with high traffic volume roads, priority for mitigation should be directed towards predators of small mammals and birds (Rytwinski and Fahrig 2013). The maintenance of road verges as attractive habitat for small species in them should be avoided whenever possible, with special attention in areas inhabited by endangered predators. Also a measure to be considered is the implementation of better perimeter fences that prevent carnivores entering the road, though the experience shows this is a hard task in most cases.

5.5 Acknowledgements

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5.6 Supplementary material

Table 5.1. Carnivore species found in Peninsular Spain. Species present in the study area are marked with an asterisk in the first column. Conservation status in Spain obtained from Palomo et al. (2007).

	Scientific Name	Common Name	IUCN-Spain Conservation Status
Canidae			
*	*Canis lupus signatus*	Iberian wolf	Near Threatened
*	*Canis familiaris*	Dog	Domestic / Feral
*	*Vulpes vulpes*	Red fox	Least Concern
Mustelidae			
	Mustela erminea	Stoat	Least Concern
*	*Mustela nivalis*	Least weasel	Least Concern
	Mustela lutreola	European mink	Endangered
*	*Mustela putorius*	European polecat	Near Threatened
*	*Neovison vison*	American mink	Introduced
	Martes martes	Pine marten	Least Concern
*	*Martes foina*	Stone marten	Least Concern
*	*Meles meles*	Eurasian badger	Least Concern
*	*Lutra lutra*	European otter	Least Concern
Ursidae			
	Ursus arctos	Brown bear	Critically Endangered
Herpestidae			
	Herpestes ichneumon	Egyptian mongoose	Least Concern
Viverridae			
*	*Genetta genetta*	Common genet	Least Concern
Felidae			
*	*Felis silvestris*	Wild cat	Near Threatened
*	*Felis catus*	Cat	Domestic / Feral
	Lynx pardinus	Iberian lynx	Critically Endangered

Chapter 6

Do roadkill patterns reveal predator-prey relationships?

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Abstract

Roads are widespread all over the world and increasing, and they are responsible for multiple effects of vertebrates, being direct mortality by vehicle collision the most conspicuous one. Casualties are often cluster around specific locations or times, roadkill hotspots. One important factor that influences the roadkill is the attraction of species to roads due to resource availability. As road verges can sustain population of small mammals, we study the effect of predator-prey interactions that may underlie the general roadkill pattern. We test three predictions: Prey roadkills will be more abundant than carnivore ones because of their higher abundance; Carnivore spatial hotspots will overlap those of their prey; and carnivore temporal trends will be similar to those of their prey. We surveyed three motorways and obtained vertebrate roadkill data from two different sources: a database of removed carcasses and a roadkill monitoring program. We analyzed the spatial coincidence of prey and predators by a modified t-test with Dutilleul correction, and the temporal coincidence by GLMs, controlling for traffic volume. We found a total of 1330 carcasses, of which 1037 were detected during monitoring surveys, mainly prey species, and 293 come from the database, mainly carnivores. Each species group showed particular roadkill patterns, but there were some coincidences. We found significant spatial correlation between medium carnivores and birds and small mammals, and between cats and lagomorphs. In the temporal analyses, there was also a positive significant effect of prey abundance in medium carnivores and cats. No correlation was found for foxes. These results point to an effect of prey abundance in the roadkill patterns of some carnivores, especially those more related to live prey hunting. We propose that mitigation measures aimed at reducing roadkills of medium carnivores should take into account prey populations in the proximity of roads.

6.1 Introduction

Road mortality is the most direct and visible effect of roads on ecosystems and one of the main sources of vertebrate mortality from anthropogenic activities (Forman and Alexander 1998, Trombulak and Frissell 2000, Underhill and Angold 2000). Roads are a perturbation source extended all over the world and an increase of 60% in road network is expected by year 2050 (Dulac 2013). Thus, mitigation measures for their pervasive effects are urgent and roadkill is among the priorities in mitigation for the road management companies (Ruiz-Capillas et al. 2013a). Due to their importance, many studies have addressed this issue and identified several key factors related to traffic mortality (see review in Gunson et al. 2011).

Roadkill affects many vertebrate species, from amphibians to large mammals or nocturnal raptors (Forman et al. 2003, Gunson et al. 2011), and can be a threat to population survival (Fahrig et al. 1995, Mumme et al. 2000, Nielsen et al. 2006), being a risk sometimes higher than barrier effect and fragmentation (Jackson and Fahrig 2011). However, the consequences of roadkill are not equal for all species. The better we understand the drivers of roadkill, the more effective mitigation measures will be implemented. Life history traits and behavior will determine the extent of the effects and the exposure of individuals to traffic (Jaeger et al. 2005, Rytwinski and Fahrig 2012). Casualties do not occur at random in space, but clustered at some points of the road network (Clevenger et al. 2003, Malo et al. 2004b). Some studies relate these so called roadkill hotspots with habitat characteristics, like wetlands or patches of preferred habitat, or with road attributes, like road width and posted speed (Ramp et al. 2005, Farmer and Brooks 2012, Barthelmess 2014). They also tend to be clustered around certain

seasons in the year, usually related to species phenology (Smith-Patten and Patten 2008, Grilo et al. 2009). Other important factor is the attraction of some species to roads due to resource availability, like salt or food (Forman et al. 2003, Gunson et al. 2011).

Road verges can be habitats for dense population of small mammals (Bellamy et al. 2000, Sabino-Marques and Mira 2011, Ruiz-Capillas et al. 2013c), and their populations can attract predators to the infrastructure (Barrientos and Bolonio 2009). Wild populations in verges are subjected to traffic mortality, probably in an amount roughly proportional to their abundance (Adams and Geis 1983, D'Amico et al. 2015), creating a regular source of carrion, that is also a resource for many generalist carnivores. In this context, areas with higher intensity of prey roadkills can be expected to be also related to higher carnivore mortality. Factors that promote carnivore roadkill are essential for the design of preventive and mitigation measures, and among them the attractors for carnivores to roads should be a priority.

Although some multispecies studies have been done (Farmer and Brooks 2012, Teixeira et al. 2013, D'Amico et al. 2015), the majority of the studies focused on specific species or taxonomically related groups and often interspecies relationships have been neglected. In this study, we contribute to the general knowledge of roadkill factors by focusing on the predator-prey interactions that may underlie the general roadkill patterns. Predator-prey interactions shape communities and play a central role in population abundance and distribution (Barbosa and Castellanos 2005, Mortelliti and Boitani 2008), thus we expect they are also reflected in the traffic mortality patterns. We use data from vertebrate species, including reptiles, small mammals and birds, to determine temporal and spatial

hotspots and relate them to the carnivore roadkills. To do this, we characterize the roadkills of three motorways and test three predictions: 1. Roadkills of prey species will be more abundant than carnivore roadkills as a reflection of their abundance in the landscape, 2. Carnivore spatial hotspots will overlap with those of their prey , and 3. Carnivore temporal trends will be similar to those of their prey.

6.2 Methods

6.2.1 Study area

The study area was located in Central Spain, in the provinces of Ávila and Segovia, and occupies an area of c. 40 x 55 km. The climate is continental Mediterranean, with cold winters and dry summers. The landscape consists in agricultural fields in the north and open woodlands in the south used for cattle ("dehesas"). In addition, there are several patches of natural vegetation and areas of conservation interest, included in Nature Network 2000. The 80% of the total length of surveyed motorways runs adjacent to a Nature Network 2000 area.

We surveyed three motorways stretches for our study (Fig 6.1): AP-6 with a total length of 30 km (milestone 80 to 110) and an average daily traffic (ADT) during our study period of 17318 vehicles day^{-1}; AP-61 with a total length of 27 km (milestones 61-88) and ADT of 6507 vehicles, and AP-51 with a total length of 23 km (milestones 82-105) and ADT of 8080 vehicles. Motorways AP-61 and AP-51 had a constant traffic flow all year round, and AP-6 had a large peak during summer months and two smaller peaks on Easter and Christmas holidays.

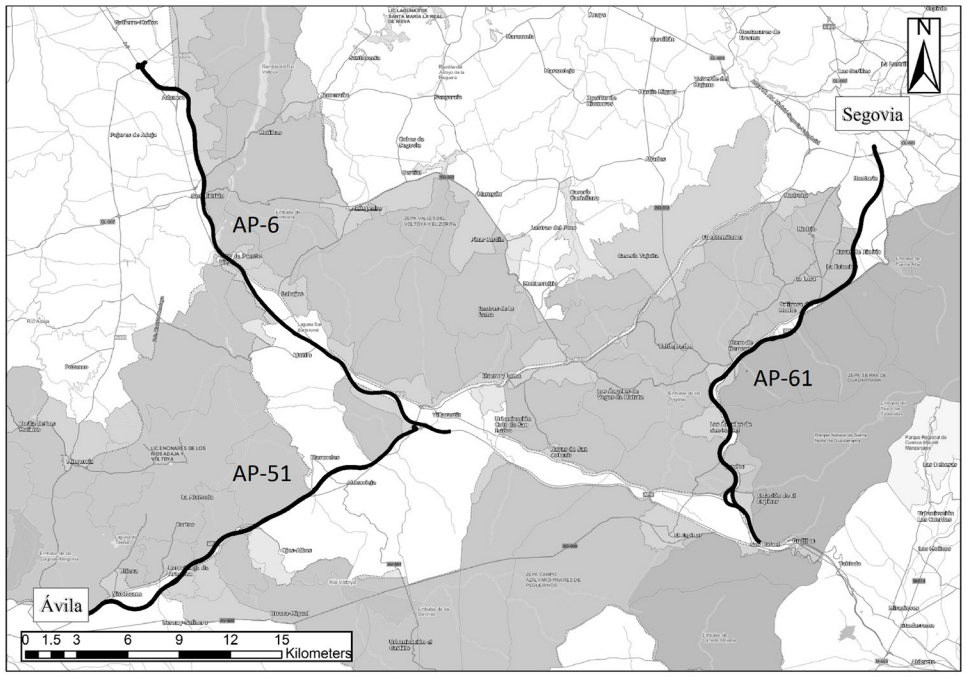

Figure 6.1. Study area map. Black lines are motorways surveyed during the study. Shadowed areas correspond with Natura Network 2000 areas.

6.2.2 Data collection

We obtained vertebrate roadkill data from two different sources: a database of removed carcasses managed by the motorway company responsible for maintenance and a roadkill monitoring program specifically done for this study.

The database was provided by the company responsible for the maintenance of the motorway (Iberpistas S.A.). This database contained all the carcasses collected by motorway workers over a 5 year period, from 2007 to 2011. Motorway workers travel the motorways two or three times a day and remove any carcass found in the traffic lanes that is big enough to be considered a safety hazard.

Usually, this means that they recorded dead animals that were the size of a stone marten or larger.

Additionally, we carried out a vehicle monitoring survey to complement the database with carcasses found on the verges and those of smaller size. These surveys were done once a month for two years, from December 2009 to November 2011, from a vehicle travelling at a constant speed of 30 kph along the hard shoulder of the motorway. The car was occupied by two people, a driver and a dedicated observer, and both directions of the motorways were surveyed. Surveys were regularly done early in the morning and the three motorways were surveyed in three consecutive days to avoid bias due to hour or meteorological conditions. When we detected a carcass, we identified it at lower taxonomic level possible and recorded the position with a GPS device. Due to traffic flow and carcass deterioration, it was not possible to identify small carcasses at species level. We recorded data from all terrestrial vertebrates except amphibians, as their small size makes them very difficult to detect from a moving vehicle.

6.2.3 Data Analysis

To deal with the uncertainty of species identification by non-trained motorway employees and carcass degradation, and to unify results from both data sources, we pooled data into similar taxonomic groups for both, prey and carnivores. Prey groups were form by lagomorphs (European rabbits and hares), birds (Passeriformes and pigeons), reptiles (snakes and lizards) and small mammals (rats, mice and voles). Carnivore groups were form by red fox (*Vulpes vulpes*), dogs, medium carnivores (medium size mustelids like stone marten, badger and polecats, and genets), and cats (including both feral and wildcats).

First of all, we present a description of data as proportions of roadkilled taxa in each motorway for prey and predator groups, and analyze differences between motorways by Chi-squared tests.

A. Spatial analysis

For spatial analyses, motorways were divided into stretches of 1 km and we calculated the sum of carcasses found in each km for each group. Roadkill hotspots were defined following Malo et al. (2004b). This method compares the number of roadkills found in each motorway stretch with those expected under a random situation, following a Poisson distribution. Each kilometer with a number of roadkills higher than the expected value for that group is defined as a roadkill hotspot.

The correlation between predator and prey roadkills was evaluated by a modified t-test for spatial data with Dutilleul correction (Dutilleul et al. 1993, Legendre and Legendre 1998, Fortin and Dale 2005). Spatial data may present spatial autocorrelation that needs to be controlled for in the statistical test to avoid bias in the estimation of correlation coefficients and variances (Dutilleul et al. 1993). The modified t-test calculate the significance of the correlation after correcting the degrees of freedom based on the extent of the spatial autocorrelation, estimated by Moran's I to (Rosenberg and Anderson 2011). The number of distance classes used for Moran's I were obtained using the Sturge rule (Legendre and Legendre 1998). As data were analyzed in 1 km stretches, we assigned the same UTM coordinates to all casualties in the same kilometer.

This spatial correlation analyses were done in PASSaGE 2 software (Rosen-

berg and Anderson 2011).

B. Temporal analysis

We also computed the average number of casualties per month to check for temporal trends in roadkill. The relationship between the temporal variation in carnivore and prey roadkills was tested by GLMs, using prey roadkill as explanatory variable and controlling for traffic volume and Motorway, to avoid a possible confounding effect of these variables in the results, as traffic volume presented intra-annual variation in only one of the three motorways. The general form of the GLM model was: "Carnivore roadkills (month average) = Prey roadkills (month average) + Traffic volume + Motorway". Model assumptions were checked in the residuals (Zuur et al. 2013).

GLM models were done in R 3.1.1 (R Core Team 2014).

6.3 Results

6.3.1 Roadkill data

We found a total of 1330 carcasses, of which 1037 were detected during monitoring surveys and 293 come from the database. 38 carcasses from the monitoring survey and 54 carcasses from the database belonged to species with very few records, such as wild boars, raptors and otters, or could not be identified, and therefore, they were not used in the analyses. All prey carcasses were detected during the vehicle survey (n = 961). Of these, 73.5% were found in the AP-6 (n = 706),

and lagomorphs were more than 40% of the total (n=394), followed by birds (n= 256), reptiles (n = 185), and small mammals (n = 125) (Fig 6.2a). Predator carcasses (n = 277) came mainly from the database of the company (86%) except for the smallest carnivores: medium carnivores and cats, that were also encountered during vehicles surveys (aprox. 40% and 50% of data for these groups, respectively, came from the vehicle surveys). Off the total data, 60% of predator carcasses were found in the AP-6 motorway, being foxes more than 50% of the total (n = 143), followed by dogs (n = 62), medium carnivores (n = 45), and cats (n = 27) (Fig 6.2b). The average of roadkilled prey found each month (one day survey) in 1 km is shown in Table 6.1. No average was calculated for predator roadkills, as data came from different sources.

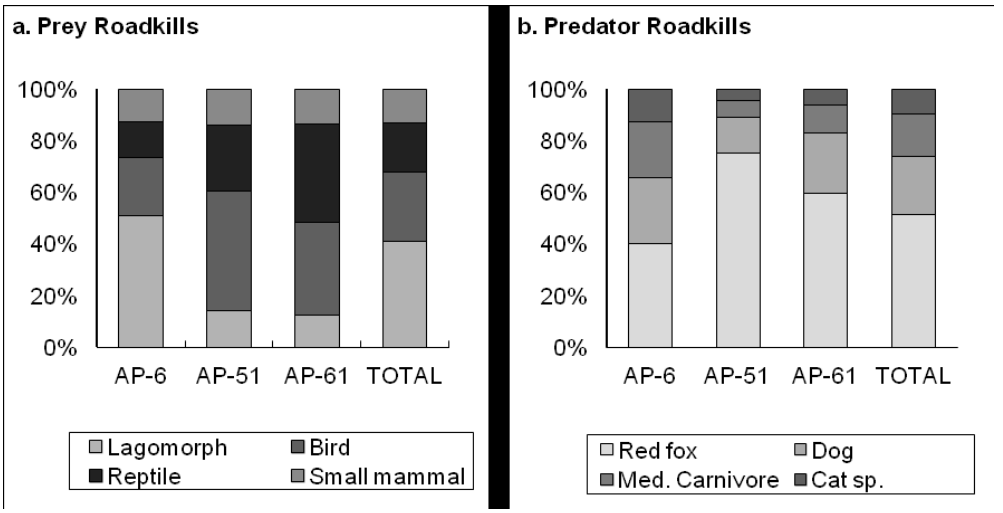

Figure 6.2. Percentages of the roadkilled taxa in each motorway, divided into prey (a) and predators (b).

Table 6.1. Number and location of the roadkill hotspots in each motorway.

Taxón	AP-6		AP-51		AP-61	
	N	**Km**	**N**	**Km**	**N**	**Km**
Prey						
Lagomorph	4	81; 101; 106; 108-109	0	-	2	83; 85
Bird	2	91-92; 106	2	88; 94	2	66;70-71
Reptile	2	94; 101	1	94	2	76;83
Small mammal	3	91;98;104	0	-	2	69;71
Predator						
Red fox	1	86	1	103	1	77
Dog	1	82	0	-	1	87-88
Med. Carnivore	4	82;87;98;102	0	-	0	-
Cats	1	81	0	-	0	-
Total	6	81;91;98-99; 101;106;108	3	88;94;101	3	66; 69-71;83

The chi-squared test revealed significant differences in the proportion of roadkilled taxa in each motorway for both groups, prey (χ^2 = 133.02, df = 6, p < 0.001) and predators (χ^2 = 25.60, df = 6, p < 0.001). Among prey, the residual table for the chi-squared indicated that lagomorphs were more abundant than expected in AP-6 and less abundant in AP-51 and AP-61. Birds showed the opposite pattern, being more abundant than expected in AP-51 and AP-61 and less in AP-6, and reptiles were more abundant in AP-61 and less in AP-6. Small mammals showed similar proportions. For predators, the residual table showed more dogs, cats and medium carnivores, and less foxes than expected in AP-6, while AP-51 presented more foxes and less dogs, and AP-61 had less medium carnivores and cats, and a higher proportions of other species.

6.3.2 Spatial analysis

The analysis of the number of roadkills in each kilometer stretch revealed several locations in the motorways were the number of carcasses was higher than expected (i.e. roadkill hotspots). When analyzed the distribution of each taxa, we found that roadkill hotspots were different for each species (Table 6.1). For the total data, we found six hotpots in AP-6, three in AP-61 that included a stretch of three consecutive kilometers, and two in AP-51 (Table 6.1). In the AP-6 the general roadkill hotspots were determined mainly by lagomorphs and birds hotspots. In the AP-61 and AP-51 they were determined mainly by birds and reptiles or small mammals (Supplementary Figure 6.4 for prey roadkill hotspots). We also found some roakills hotspots for red foxes and dogs in all three motorways (Supplementary Figure 6.5 for predator roadkill hotspots), but they did not coincide with those of any potential prey.

Table 6.2. Correlation coefficients and significance level obtained in the modified t-test with Dutilleul correction for spatial correlation between taxonomic groups.

	Red fox	**Dog**	**Med. Carnivore**	**Cats**
Lagomorph	0.037	0.258	0.255	0.521 **
Bird	0.184	0.211	0.245	0.285 *
Reptile	-0.190	-0.054	0.149	0.171
Small mammal	0.128	-0.003	0.367 *	0.102
Cats	0.047	0.432 ***	0.101	
Med. Carnivore	0.132	0.161		
Dog	0.164			

* $p < 0.05$: ** $p < 0.01$; *** $p < 0.001$

In the analysis of the spatial correlation between predators and prey, the t-test found few significant correlations (Table 6.2): medium carnivores were correlated with birds ($r = 0.31$, $p = 0.032$) and small mammals ($r = 0.37$, $p = 0.023$), and cats were correlated with lagomorphs ($r = 0.52$, $p = 0.003$). There is also a correlation between cats and dogs ($r = 0.43$, $p < 0.001$).

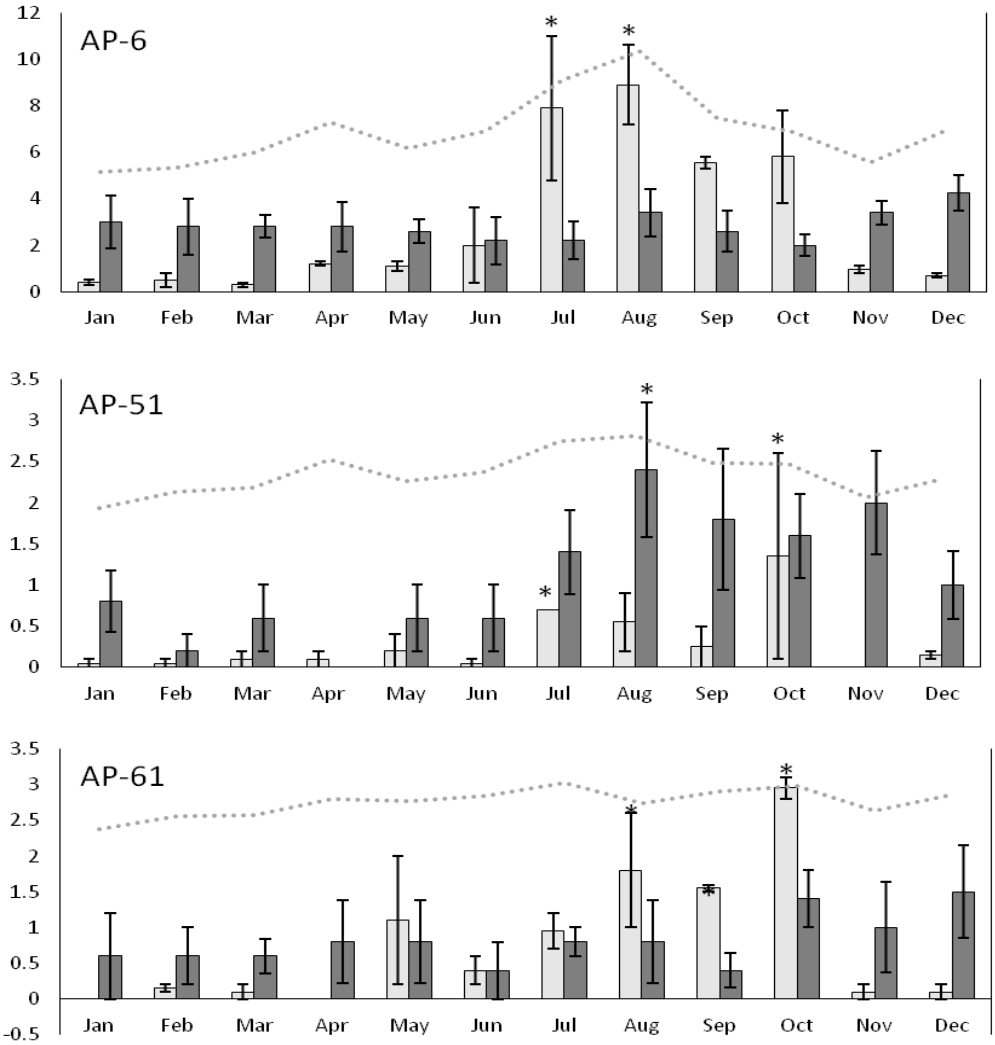

Figure 6.3. Mean number (±SE) of prey (light grey) and predators (dark grey) found per month on each motorway. Asterisks indicate months with very high mortality, when average mortality of a particular month was at least twice as great as the annual average mortality. Dashed line represent the average daily traffic (ADT).

6.3.3 Temporal analysis

Regarding the temporal aspect, most of the roadkills recorded during the vehicle survey occurred during the summer months, especially for prey species, with a second peak around early autumn (Fig. 6.3). Roadkills of the carnivores did not show a marked temporal pattern, being different in each motorway, with a peak around august and another one in the last months of the year (Fig. 6.3). All taxonomic groups showed the highest temporal variability in the AP-6 motorway, except for reptiles that showed a second peak in the AP-61 in October and foxes, that showed a peak in summer in AP-51. Peaks in roadkill abundance occurred from July to October in all prey species. Carnivores presented different peaks depending on the species, with peaks in August and December for foxes, December to February for dogs, March and August for medium carnivores, and several peaks for cats Temporal patterns of each taxonomic group are in the Supplementary Figures 6.6 and 6.7 for preys and carnivores, respectively.

When analyzed the season pattern, there was a positive significant effect of prey abundance in medium carnivores and cats, and a negative relationship between prey roadkill abundance and dog roadkills (Table 6.3). No relationship with prey was found for foxes. In these models, traffic volume presented a positive coefficient for all species, although it was only significant for foxes when birds were included as prey ($t = 2.05$, $df = 31$, $p = 0.048$).

6.4 Discussion

We found a high incidence of roadkills in our motorways, with spatial and temporal patterns for the different groups, among which some interesting correla-

Table 6.3. Coefficients and their significance in the temporal models for carnivores and prey.

	Red fox		Dog		Med. Carnivore		Cats	
	β ± SE	**p**	**β ± SE**	**p**	**β ± SE**	**p**	**β ± SE**	**p**
Lagomorph	-0.01 ± 0.01	0.612	-0.03 ± 0	< 0.001	0.02 ± 0	0.001	0 ± 0	0.354
Bird	-0.02 ± 0.02	0.326	-0.04 ± 0.01	< 0.001	0.02 ± 0.01	0.012	1.51E-02	0.003
Reptile	-0.01 ± 0.02	0.572	-0.04 ± 0.01	0.003	0.03 ± 0.01	0.011	1.23E-02	0.050
Small mammal	-0.02 ± 0.03	0.486	-0.07 ± 0.02	< 0.001	0.05 ± 0.01	< 0.001	8.86E-03	0.267

tions between predator and prey species arise. Although correlation between prey and carnivore roadkills was not as general as expected by our predictions, some patterns point to a parallelism is abundance between prey and some carnivores, like mustelids (our medium carnivore group).

The proportion of each taxonomic seems to reflect the wildlife community (Ruiz-Capillas et al. 2013a). Areas with more dog roadkills are located near urban zones (Fig. 6.4), where we expect a human tolerant community of generalist species. And areas with more medium carnivore roadkills and those located in forested and undisturbed areas (Fig. 6.5), where the community is less tolerant to disturbance. Other species are generalists and show high tolerance to human disturbance and fragmented habitat in agricultural landscapes, appearing in all the study area, like rabbits and red fox (Baker and Harris 2007). In this sense, we could use the abundance of roadkills of some of the species as an indicator of areas where to expect roadkills of species with similar habitat requirements (dogs for urban tolerant communities, medium carnivores for wild communities). These proportions are useful even if data do not reflect the total amount of actual roadkills, as many small carcasses were probably undetected because of the sampling method, fast degradation, or removal of carcasses by scavengers (Santos et al. 2011, Teixeira et al. 2013a). Assuming that we detected carcasses as much as one week old (that is a very optimistic assumption), actual roadkill numbers will be at least four times higher.

The temporal and spatial patterns are essential for the implementation of effective mitigation measures (Lesbarrères and Fahrig 2012). Many of the spatial roadkill hotspots detected in this study were located where the infrastructure crosses rivers or forested areas, that are both corridor for fauna in Mediterranean

landscapes (Virgós 2001, Matos et al. 2009). However, some spatial correlations were detected. The most specialist carnivores in our study area –medium carnivores, mainly mustelids–, were correlated with their prey, small mammals, and the same occurred for cats and their prey, birds and lagomorphs (Padial et al. 2002, Biro et al. 2005, Lozano et al. 2006). These results support the hypothesis that areas of high prey abundance result on carnivore mortality, as already suggested for owls (Gomes et al. 2009, Carvalho and Mira 2011). On the contrary, the most opportunistic carnivore, the red fox, appeared in different locations than its prey, probably due to it feeds on garbage or anthropogenic resources near rural zones (Baker et al. 2007, Bateman and Fleming 2012).

The temporal trends in roadkills found for each taxonomic group were more related to their phenology than to changes is traffic volume inside the same motorway (Clevenger et al. 2003, Smith-Patten and Patten 2008, Grilo et al. 2009). Animals are more active during breeding and juvenile dispersal seasons, and that increase in activity is reflected by a higher roadkill rate. This period is similar for all prey species, particularly during summer months, when juveniles disperse from their parental territories (Blanco 1998, Erritzoe et al. 2003, Villafuerte and Delibes-Mateos 2007). Reptiles, mainly snakes, also use roads for thermoregulation until October (Bonnet et al. 1999, Garrah et al. 2015), explaining the temporal pattern of this group.

Among carnivores the temporal peaks were lower but they still presented a temporal pattern. For most species, the peaks coincided with mating and dispersal periods (Blanco 1998). Once again, those species that hunt live prey area the ones correlated to prey. In the case of medium carnivores, the peak of dispersal coincided with the one of the prey, both small mammals and lagomorphs. It is

probable that inexperienced juveniles are attracted by the high prey density in motorway verges during this time, and therefore being highly exposed to traffic (Baker et al. 2007). Something similar can be applied to cats and birds, although this interpretation should be made with caution because in our data feral and wildcats were pooled together.

The temporal and spatial correlation between medium carnivores and small mammals, and cats and birds, can be taken as a clear sign of predator-prey dynamics that are being reflected in the roadkill abundance.

In our study, more than 65 % of all carcasses were found in the AP-6 motorway. AP-6 has a traffic volume two or three times higher than the other motorways that can contribute to more roadkill (Lodé 2000, Saeki and Macdonald 2004), although this issue is controversial and there is no clear effect of traffic volume in all circumstances (Grilo et al. 2015). Another factor can be differences in habitat between the motorways. Habitat around AP-6 is predominantly agricultural, and habitat around AP-51 and AP-61 is more forested and the main activity is traditional cattle grazing in pastures with scatter trees ("dehesas"). Although wooded traditional pastures are richer in species diversity, road verges are an important landscape feature for small mammals and carnivores, that use them as habitat or corridor when they are located in a hostile matrix, as is the case for the field crops (Bellamy et al. 2000, Frey and Conover 2006)..

Another result than can be derived from our study is the comparison between data provided by the motorway companies and those taking by specialized researchers. The specialized survey, although less frequent, provided essential data that would be lost otherwise. Also, database from the motorways proved reliable for big species, such as dogs and foxes, but not for smaller ones. Also, carcasses of

small size were not identified in many cases by motorway workers. As these regular surveys are a good source of data, the quality of the records may be improved by the development of standardized protocol to record roadkills from a scientific perspective, and by training the people working for the motorway companies on species identification.

In summary, temporal and spatial patterns are characteristics of each taxonomic group and mitigation measures should be adapted to the variability presented by each species to be effective (Teixeira et al. 2013b, Barthelmess 2014, Garrah et al. 2015). For those species more related to live prey hunting, hotspots of prey roadkills can be related to those of carnivore roadkills in space and time. Mitigation measures aimed at reducing roadkills of medium carnivores, and probably wildcats, should take into account prey populations in the proximity of roads. An interesting measure would be to limit the prey populations by making verges less attractive to them, for example, removing shrubs that are attractive to birds, or lowering plant cover to reduce small mammal and rabbit populations. Together with this, provide alternative habitat of good quality away from traffic when infrastructure is surrounded by a hostile matrix should attract some populations away from risky areas. If alternative habitat with no disturbance is available, it will probably be selected instead of motorway verges (Planillo et al. 2013).

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6.6 Supplementary material

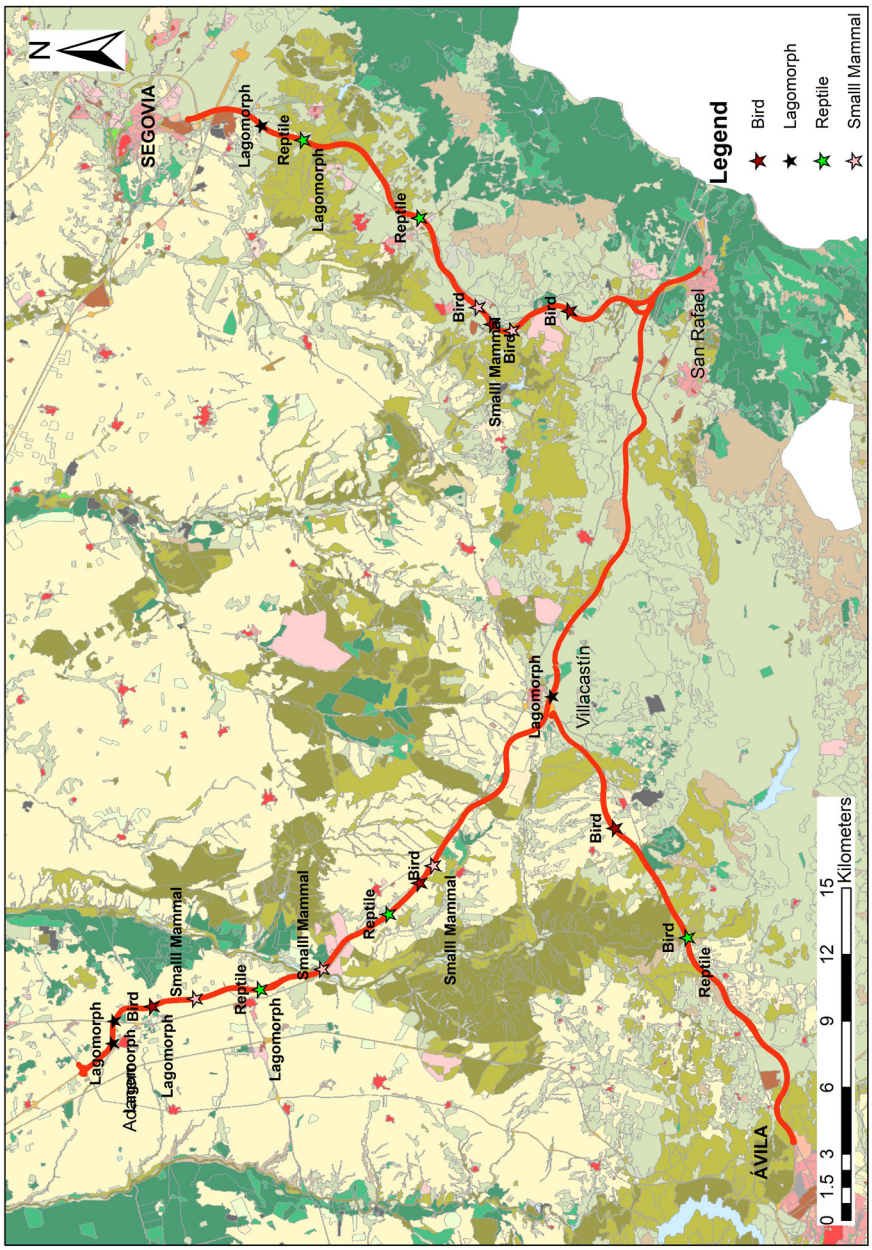

Figure 6.4. Prey roadkill hotspots in the study area.

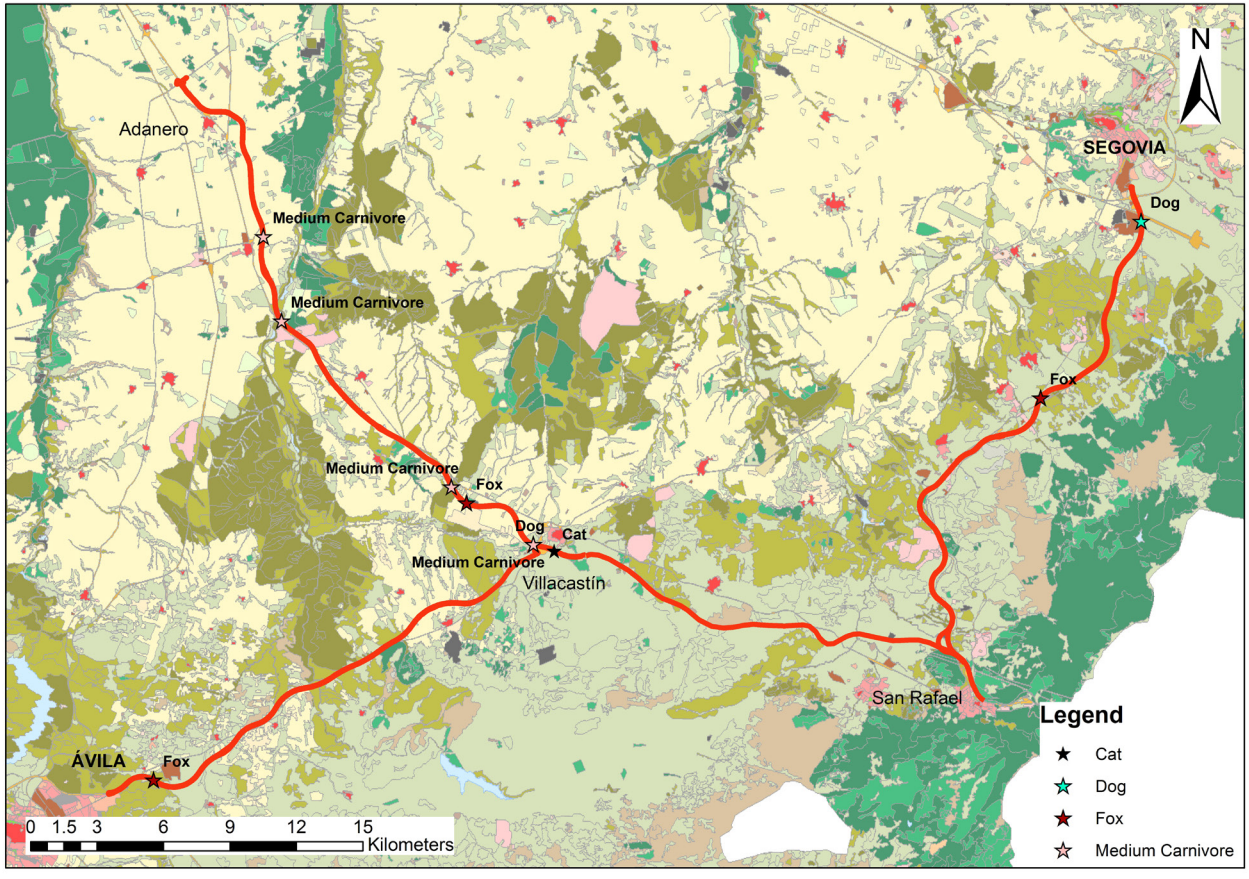

Figure 6.5. Predator roadkill hotspots in the study area.

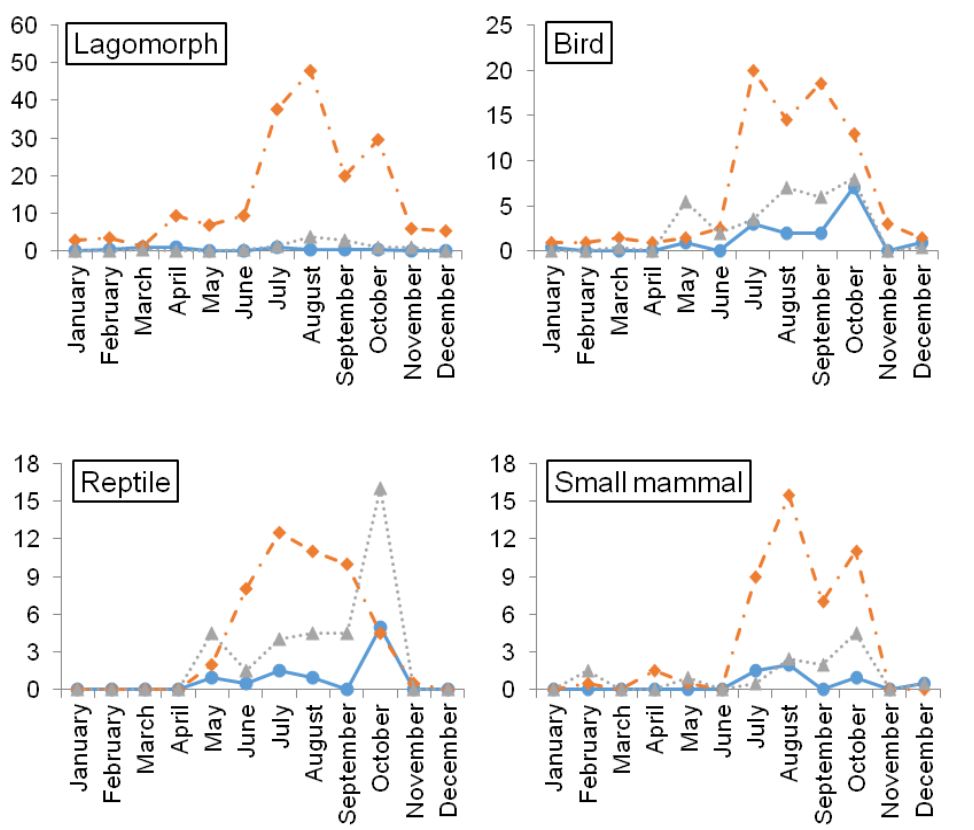

Figure 6.6. Temporal patterns of prey roadkills (mean number of prey detected each month).

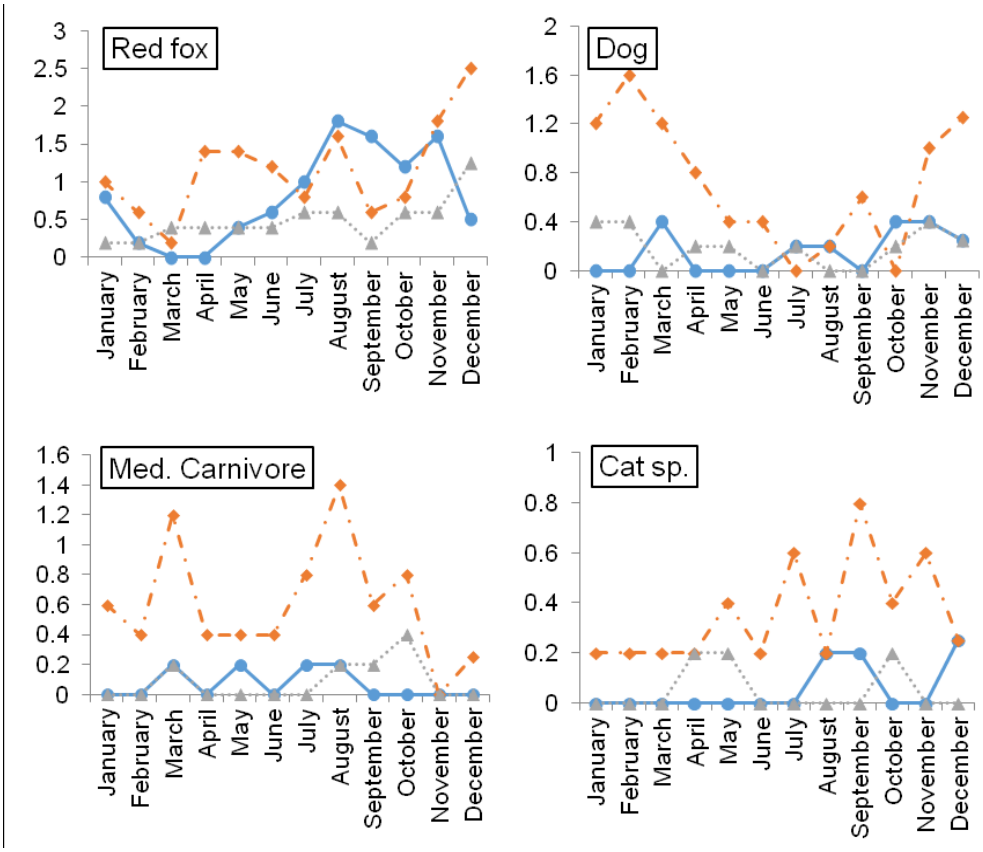

Figure 6.7. Temporal patterns of predator roadkills (mean number of prey detected each month).

Chapter 7

Discusión general

Al inicio de la tesis, hemos establecido que las carreteras alteran el hábitat de su entorno, disminuyendo su calidad para algunas especies y favoreciendo poblaciones de otras (Forman et al. 2003). Durante los cinco capítulos centrales se pretendía profundizar en los efectos que dicha alteración provoca en el ecosistema, a través de cambios en las poblaciones de presas y de efectos en cascada en las poblaciones de depredadores, reflejadas en su uso del hábitat o en su abundancia.

En relación con la especie presa, el conejo, en los Capítulos 2 y 3 se ha analizado su respuesta a las autopistas, tanto en función de la distancia como de las características de los taludes y el paisaje. El conejo es una especie tolerante a la perturbación humana y es frecuente en zonas rurales e incluso, parques urbanos (Garden et al. 2006, Baker and Harris 2007). En nuestro análisis de abundancias respecto a la distancia a la fuente de perturbación, la autopista (Capítulo 2), destaca precisamente este hecho, que es tolerante a la perturbación, pero no selecciona activamente zonas alteradas cuando dispone de hábitat natural. Este resultado

indica que, si bien las poblaciones de conejo parecen ser capaces de sobrevivir en zonas de mucho tráfico, también deben sufrir los efectos negativos (Capítulo 3), como la mortalidad o el aumento de estrés por la proximidad de la autopista (Strasser and Heath 2013, Navarro-Castilla et al. 2014).

Esta idea se ve reforzada en el Capítulo 3, que determina que los taludes que favorecen mayores abundancias son aquellos más anchos y localizados en zonas más mediterráneas. La anchura de los taludes ya ha sido destacada en otros estudios como uno de los factores importantes, debido a que delimita la cantidad de hábitat disponible (Bellamy et al. 2000, de Redon et al. 2015). El clima mediterráneo es el propio del conejo (Blanco 1998), pero en nuestro estudio también coincide con la parte más alterada, donde las posibilidades de los conejos de establecerse fuera de los taludes son escasas, y donde los taludes ejercerían como refugio para las poblaciones. Un refugio que a su vez genera mortalidad de la especie y es conocido y explotado por los depredadores (Capítulo 5).

Cabe destacar especialmente Capítulo 3 que la importancia de las variables asociadas a la infraestructura es mucho mayor que la asociada a características del medio, en un estudio que incluye un amplio rango de variabilidad ambiental. En otras palabras, la variabilidad en la abundancia de conejos en los taludes responde a las características de la propia infraestructura y, por tanto, no se trata de un efecto secundario con el que haya que convivir, sino que es potencialmente manejable mediante cambios en las características de los taludes. Por ejemplo, reduciendo el ancho del talud se minimizan las poblaciones de conejo, frenando su dispersión por el paisaje y suavizando el efecto cascada sobre los depredadores (Capítulo 5).

En el Capítulo 4 se observa que la relación de las aves rapaces con las carreteras y autopistas se puede dividir en tres categorías: especies que evitan la proxi-

midad de infraestructuras en cierto grado, especies cuyo uso del hábitat viene determinado por la abundancia de presas, y especies que seleccionan positivamente las carreteras y/o autopistas. Este último caso se trata de especies generalistas de mediano tamaño, como los milanos, que recorren las carreteras en busca de carroña o presas y pueden reaccionar rápidamente frente a vehículos (Meunier et al. 2000, Blumstein et al. 2005), pero que identifican la carretera en sí como el elemento clave. Se trata de una atracción pura de las infraestructuras. Un caso más complejo es el de las especies que deciden su uso del hábitat en función de la abundancia de presas, sin un efecto aparente de la infraestructura. Si estas especies establecen su área de campeo en zonas donde las presas se encuentran principalmente en los taludes, serán atraídas también hacia el tráfico. En función de los resultados de los Capítulos 2 y 3, para depredadores de conejo, esto sería especialmente esperable en zonas con taludes anchos que atraviesen una matriz de paisaje muy manejado. Es decir, en aquellas zonas donde las rapaces ya están sometidas a perturbación y donde sus poblaciones pueden ser más vulnerables.

El efecto cascada de la abundancia de presas en la mortalidad de depredadores se estudió en los Capítulos 5 y 6, con enfoques ligeramente distintos, pero resultados complementarios. En el caso de los carnívoros (Capítulo 5) cabe destacar que se encuentra menos diversidad de respuestas frente a las autopistas que en las rapaces, y en general, tienden a aumentar su abundancia junto a la carretera. Esta diferencia probablemente esté relacionada con que las especies de carnívoros observadas en el área de estudio son de mediano tamaño y generalistas (Blanco 1998), característica que ya en las rapaces se relacionaba con una mayor abundancia junto a las infraestructuras. Por otra parte, se encontró una tendencia positiva entre las abundancias de mustélido y conejo junto a la autopista, aunque no fue así para el zorro, de forma similar a los resultados obtenidos al estudiar

los patrones de atropello en el Capítulo 6. En ambos casos, la mortalidad de los carnívoros estaba relacionada con su abundancia.

De forma similar, al analizar los patrones temporales y espaciales de atropellos de presas y carnívoros en el Capítulo 6, se obtiene que los patrones de los zorros no se correlacionan claramente con ninguna presa, pero en el caso de los carnívoros medianos (principalmente mustélidos), vuelve a encontrarse una correlación espacial y temporal con sus presas. Hay que tener en cuenta que la preferencia de los carnívoros por la cercanía a autopistas puede no deberse exclusivamente a la abundancia de presas, habiendo otros factores que también favorecen la presencia de carnívoros, como una mayor abundancia de recursos en forma de basura o carroña sobre el asfalto (Clevenger and Wierzchowski 2006). Los zorros que habitan zonas alteradas se alimentan principalmente de desperdicios y carroñas, mientras que los mustélidos, aun siendo relativamente tolerantes, presentan preferencia por presa viva (Baker et al. 2007, Bateman and Fleming 2012). Mientras que los desperdicios es esperable que se encuentren distribuidos de forma más o menos homogénea a lo largo de los taludes, las presas se localizan en zonas concretas (Capítulo 3) y por tanto, el efecto cascada de mortalidad será esperable en las zonas de alta abundancia de presas para aquellos carnívoros que cazan de forma activa.

Un tema muy discutido en la literatura es el efecto del volumen de tráfico sobre la cantidad de atropellos y la respuesta de las especies a la proximidad de carreteras. El volumen de tráfico de las autopistas también ha sido un factor que ha jugado un papel central en los Capítulos 3, 4 y 6. En el análisis de los atropellos del Capítulo 6 se destaca que los números más altos ocurren en la autopista con mayor volumen de tráfico, en lugar de encontrar la relación cuadrática esperable

según la literatura (Clarke et al. 1998). No obstante, nuestros máximos de tráfico se aproximan a los máximos propuestos para las curvas de atropello de conejos y zorros, las presas y depredadores más comunes del área, por lo que no es descartable que pudiera haber una disminución de la mortalidad por atropello a mayores volúmenes (Grilo et al. 2015). Las poblaciones de conejos en los taludes son menos abundantes cuando el tráfico es muy alto (Capítulo 3), probablemente relacionado con el número de atropellos. Por otro lado, observamos que hay especies que en su uso del hábitat muestran evitación de los tramos con mayor tráfico aunque con distintos umbrales según las especies (Capítulo 4, Reijnen et al. 1996). El volumen de tráfico actúa como un filtro para las especies, alterando la composición de especies junto a las autopistas y permitiendo la supervivencia únicamente de las más tolerantes a la perturbación y a la mortalidad (Fahrig and Rytwinski 2009). Ello se traduce en que carreteras con altos volúmenes de tráfico son elementos importantes en el proceso de homogenización biótica (McKinney 2006).

Un análisis interesante que se puedo hacer de los resultados de los distintos capítulos está relacionado con la distancia hasta las que llegan los efectos negativos del tráfico. Generalmente, ésta dependen del volumen de vehículos (Reijnen et al. 1996), pero también de las características de las propias especies. Especies más grandes se ven afectadas a distancias mayores, probablemente relacionado con su mayor movilidad y mayores áreas de campeo (Fahrig and Rytwinski 2009). En el Capítulo 3, el conejo alcanza su máxima abundancia a unos pocos centenares de metros de la autopista, mientras que las abundancias máximas de carnívoros se producen en la zona más alejada.

El resultado del Capítulo 3 contrasta con el análisis realizado en al Capí-

tulo 5. En este último, al estudiar la abundancia de carnívoros a escala de paisaje, observamos que su abundancia aumenta en las zonas próximas a autopistas. Aunque aparentemente contradictorios, ambos resultados son complementarios y conviene analizarlos en su conjunto, teniendo en cuenta el factor del hábitat. La autopista analizada en el Capítulo 2 atravesaba una zona que estaba sometida a un manejo extensivo y con varias manchas de vegetación natural. En el caso del Capítulo 5, el área de estudio se caracteriza por una mayor transformación y manejo, especialmente en las zonas agrarias. Los taludes de las autopistas cobran mucho más protagonismo cuánto más modificado esté el paisaje.

Por último, otro aspecto que se puede destacar de los resultados de esta tesis es el efecto de la escala a la hora de estudiar el comportamiento de las especies respecto a las autopistas. Los estudios locales que se realizan sin zonas control de comparación se centran en animales ya acostumbrados a la presencia de la infraestructura, por lo que a veces el comportamiento no es generalizable a la especie. En el uso del hábitat de los depredadores estudiado en los Capítulos 4 y 5, hay patrones que emergen únicamente a gran escala. La respuesta de la mayoría de las rapaces a las carreteras (Capítulo 4) se observa cuando se estudia a nivel de paisaje y es difícilmente detectable a escala local. Además de informarnos de la reacción de las rapaces frente a las carreteras, estos resultados también apuntan a que las carreteras son uno de los factores decisivos en el uso del hábitat, al ser seleccionados a grandes escalas (Rettie and Messier 2000). Por otro lado, algunos carnívoros muestran un comportamiento distinto cuando están junto a autovías. Al estudiar la relación de los mustélidos con la abundancia de conejo, a escala de paisaje no se observa ninguna, mientras que en las autopistas muestran una tendencia positiva (Capítulo 5). Como se indicaba en el Capítulo 5, la mayoría de los mustélidos encontrados fueron garduñas (Martes foina), que es una especie adaptable (Ba-

teman and Fleming 2012) y, por tanto, es difícil extrapolar el comportamiento observado en otras áreas a los márgenes de las carreteras.

Chapter 8

Conclusions

1. The European rabbit, although it is tolerant to human perturbation, prefers areas of natural habitat at a distance from the motorway instead of motorway verges.

2. The variability in rabbit abundance in motorway verges, when accounting for environmental variables, is explained by characteristics of the motorway itself, thus relatively easy to manage. In areas of good climatic conditions, rabbit populations decrease when verges are less than 10 m wide.

3. Among birds of prey species, big scavengers avoid roads, especially high traffic motorways.

4. Generalist and medium sized raptors are those that increase their abundance near motorways or select them during their habitat use, as they profit from different food resources and are tolerant to human disturbance.

5. In a parallel way, generalist and medium sized carnivores are also attracted to motorways for resources, increasing their abundance around motorways compared to control sites.

6. The cascading effect of prey abundance in verges on carnivore mortality is expected in carnivores that hunt live prey, instead of those that feed on carrion or waste.

7. Temporal and spatial roadkill patterns are species dependent and mitigation efforts should be addressed and design for target species.

8. Negative effects of motorways on vertebrates increase with traffic volume, at least until a value of 25.000 vehicles a day.

9. Observed individual behavior toward roads at local scales may not reflect species preference, thus being advisable the inclusion of control areas to evaluate the actual response of the species to traffic.

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